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# « NOYAU FERMÉ » ET « NOYAU OUVERT »

## CONTRIBUTION À L'ÉTUDE CYTOARCHITECTONIQUE DU TRONC CÉRÉBRAL ENVISAGÉE DU POINT DE VUE DU MODE D'ARBORISATION DENDRITIQUE

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### INTRODUCTION

L'intérêt du neuroanatomiste comme celui du neurophysiologiste est surtout orienté, à l'heure actuelle, vers l'étude des connexions de la substance grise grâce aux nouvelles méthodes introduites par Glees, Nauta et d'autres auteurs, ainsi que vers l'étude de la structure et de la fonction de la synapse. Par contre, on ne s'est pas beaucoup attardé sur les dendrites qui constituent une partie très importante de la cellule nerveuse. L'étude quantitative de Sholl (19) nous affirme cependant que la surface du péricaryon ne constitue qu'environ dix pour cent de la surface d'un neurone et que le reste de cette surface est occupé par les dendrites. En fait, Rasmussen (16) trouve, grâce à sa nouvelle méthode, beaucoup de boutons terminaux articulés avec les dendrites aussi bien qu'avec le péricaryon.

Pour l'étude topographique du système nerveux central on recourt habituellement, de nos jours, aux méthodes de Nissl ou de Weigert, lesquelles ne colorent que le péricaryon de la cellule nerveuse et la fibre myélinisée. Les dendrites échappent alors à notre observation. Afin de les observer nous aurons recours à la méthode de Golgi ou d'Ehrlich, grâce auxquelles Lénhossek (9), Kölliker (7), van Gehuchten (5), Cajal (2), etc. ont laissé des travaux classiques

et excellents. Mais il reste encore, nous semble-t-il, quelque chose à y ajouter.

Au point de vue fonctionnel, on admet, depuis Cajal, que les dendrites et le corps cellulaire ont une conduction axipète, c'est-à-dire conduisent les influx vers le cylindraxe. Dans ce sens il est donc nécessaire d'avoir une connaissance précise de la topographie des champs dendritiques lors de la stimulation ou de la destruction du système nerveux central par les méthodes physiologiques actuelles.

Il nous paraît donc très important d'examiner à nouveau la topographie cytoarchitectonique au moyen de la méthode de Nissl ou de Weigert en tenant compte de l'arborisation dendritique, et cela, au niveau du tronc cérébral, y compris la formation réticulée.

## Methodes

La méthode de Golgi, modifiée par Cox, est employée, en principe, pour colorer le tronc cérébral des sujets nouveaux-nés: lapin, chat et enfant. La méthode de Golgi-Cox imprègne les dendrites et le péricaryon beaucoup mieux que le cylindraxe. Comme la méthode ne donne pas des résultats costants, il nous a fallu utiliser de nombreux sujets.

Des animaux nouveaux-nés et âgés d'un mois au plus, sont tués par décapitation sous anesthésie au nembutal et leurs cerveaux sont rapidement prélevés. Dans tous les cas, le cerveau est séparé en trois parties sectionnées au niveau de l'infundibulum et de la partie supérieure de la protubérance. Dans quelques cas il est également coupé horizontalement ou sagittalement. En principe, nous avons laissé les pièces dans la solution de Cox pendant deux mois. Dans le cas où le cerveau est petit, celui-ci est plongé tout entiers sans être coupé, ce qui donne parfois de bons résultats. Après la déshydratation à l'alcool, l'inclusion des pièces est faite dans de la celloïdine. Nous avons effectué des coupes sériées d'épaisseurs variables, de 80 à 200 $\mu$. Les coupes numérotées en ordre sont mises dans une solution de 5% d'ammoniaque pendant trente minutes et ensuite dans l'hématéine de Carazzi afin de faire contraster les éléments qui n'ont pas été colorés par la méthode de Golgi. Après déshydratation et clarification, les coupes sont montées finalement sur la lame porte-objets et couvertes de baume.

Nous avons utilisé les coupes sériées de lapin, chat et homme, colorées par les méthode de Nissl et de Weigert, comme matériel de contrôle.

Pour suivre le trajet exact des dendrites et des cylindraxes, nous avons employé l'appareil d'Abbe quelque peu modifié afin de rendre possible, sous fort agrandissement, le dessin détaillé de chaque élément cellulaire.

Pour obtenir une vue d'ensemble de la disposition des dendrites, nous avons eu recours aux photos sériées; les photos d'une coupe sont faites en série, avec un faible agrandissement du microscope (objectif 10 X, oculaire 5) et successivement d'un champ à l'autre. Elles sont ensuite juxtaposées suivant l'ordre. On met un papier transparent sur la photo de coupe ainsi préparée et, en observant la section sous moyen ou fort agrandissement du microscope, il est possible de suivre le parcours des prolongements cellulaires d'une façon précise.

## Resultats

1. *Classification des neurones suivant l'aspect de l'arborisation.* — La classification des prolongements du neurone en deux groupes, cylindraxes et dendrites, est basée, depuis Cajal, sur l'étude de coupes colorées par la méthode de Golgi; les dendrites sont caractérisés par leur grand calibre et la présence d'épines sur leur surface, sauf à leur origine, tandis que le cylindraxe et ses collatérales sont plus fins que les dendrites et en général sans aucune épine le long de leur parcours.

Suivant le mode d'arborisation dendritique, on pourrait classer grossièrement les neurones en deux types: cellules multipolaires et cellules étoilées.

*a*) Les cellules multipolaires ont des dendrites généralement rectilignes (Fig. 1 *a* et *d*). Ces derniers se recourbent cependant, surtout à l'extremité du noyau, en rétrogradant vers l'intérieur. Les cellules à dendrites conglomérés sont une modification de ce type. On les trouve dans certains noyaux, par exemple le noyau externe de Monakow, l'olive bulbaire (Fig. 1 *b*), etc.

*b*) Les cellules étoilées ont des dendrites généralement courts, qui se ramifient tout près du péricaryon. Les dendrites sont plus grêles que ceux du premier type et, le plus souvent, ne se conglomèrent pas (Fig. 1 *c*). Au niveau de la formation réticulée, cette sorte de neurone existe exclusivement dans certains endroits, surtout dans les noyaux précérébelleux. En outre, nous avons remarqué quelques éléments semblables dispersés dans le raphé. Cette espèce prédomine de toutes façons dans la moitié médiane ou ventromédiane de la formation réticulée.

Il ne semble pas y avoir de parallélisme entre le volume du péricaryon et la longueur des dendrites. S'il est très fréquent que des cellules de grande taille aient des dendrites longs, celles de taille moyenne ou petite peuvent aussi posséder des dendrites longs et épais. En fait, au sens strict, il est impossible de connaître la vraie longueur des dendrites. Dans la majorité des cas, en effet les prolongements d'une cellule, jusqu'à leur extrémité, ne sont pas tous visibles sur une seule coupe et il nous est alors pratiquement impossible, du moins à l'heure actuelle, de connaître tout son trajet.

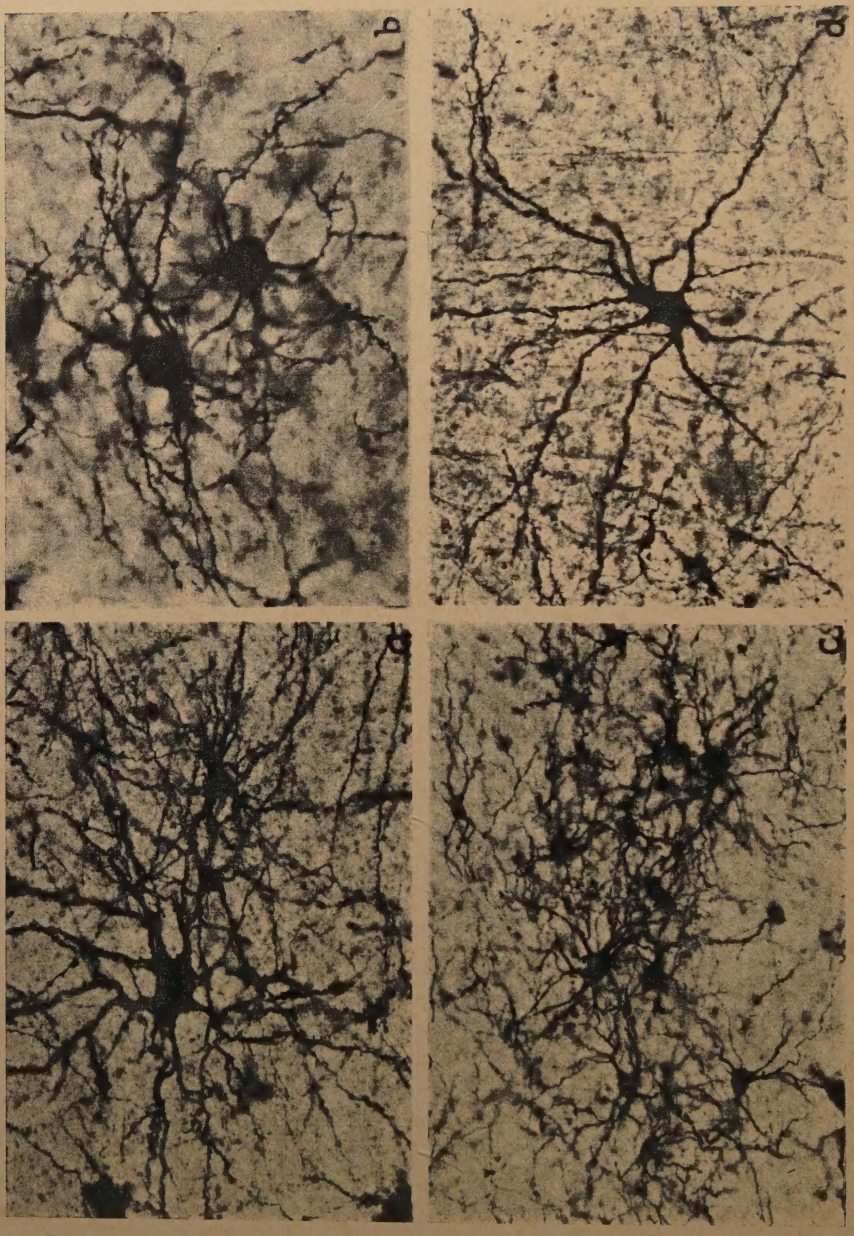


Fig. 1.

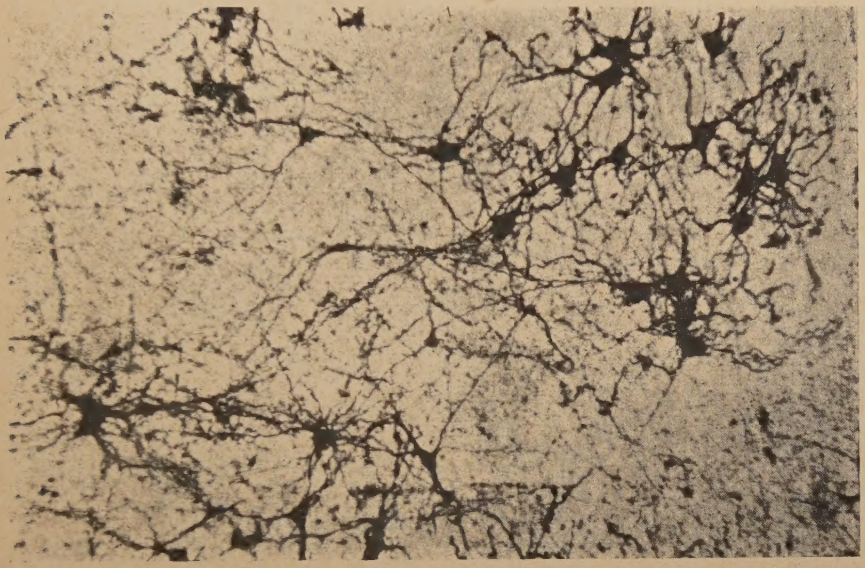

Fig. 2. – *Echange de dendrites entre noyau du nerf hypoglosse (droit supérieur) et la formation réticulée (gauche inférieure).*

Des dendrites du premier pénètrent dans la dernière, et inversement. Chat, × 50.

2. *Les noyaux moteurs du tronc de l'encéphale.* — *Le noyau du nerf hypoglosse* a un contour net sur les coupes de Nissl ou de Weigert. La préparation de Golgi nous montre que beaucoup de dendrites restent dans le foyer, tandis que certains dendrites issus des cellules ventrolatérales dépassent les limites du noyau et s'insinuent en éventail dans la partie dorsomédiane de la formation réticulée (Fig. 2), la partie ventrale du noyau dorsal du vague et du noyau intercalaire (Staderini). La plus grande longueur de dendrites externes que nous avons observée est de 600 $\mu$. Les cellules internes envoient quelques dendrites dans le noyau du côté opposé

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Fig. 1. – *Microphotographie montrant divers types de cellules nerveuses au niveau du tronc cérébral.*

*a*: grande cellule multipolaire du noyau gigantocellulaire au niveau de la moelle allongée supérieure. A côté, deux cellules multipolaires de taille moyenne. Chat, × 100; *b*: olive bulbaire. Cellules à dendrites conglomérés. Chat, × 400; *c*: noyau réticulé de la calotte protubérantielle. Cellules étoilées dont les dendrites, plus grêles et plus courts, restent habituellement à l'intérieur du noyau. Chat, × 100; *d*: cellule du raphé (au centre) qui envoie des dendrites dans deux directions. Chat, × 100.

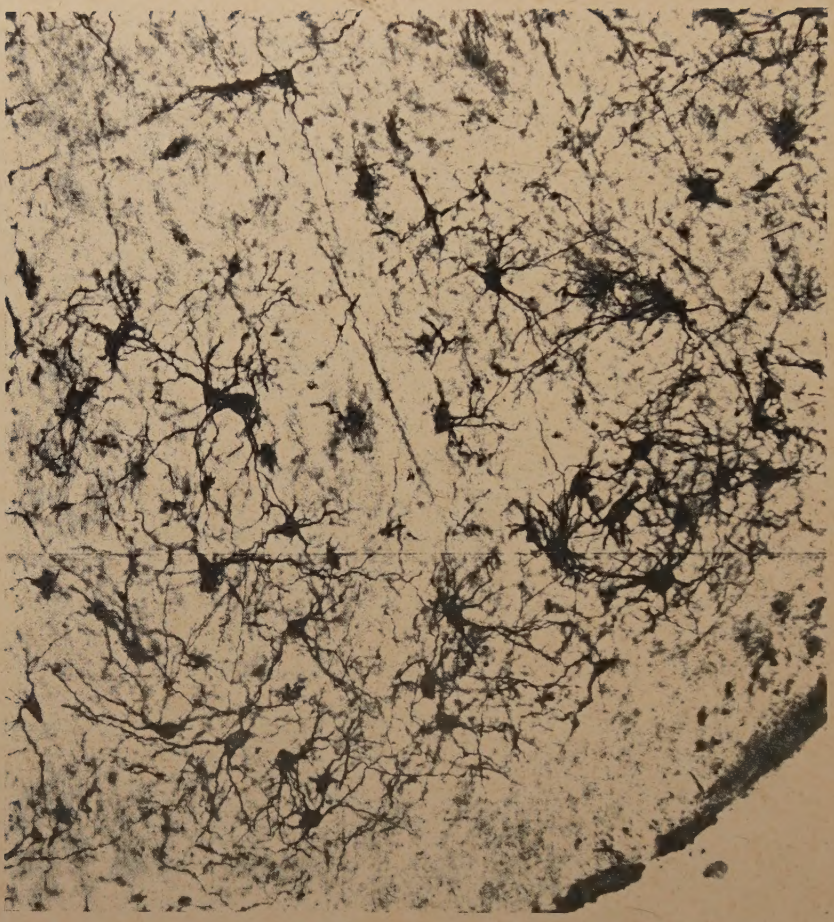


Fig. 3. – *Noyau facial à contour oval.*

Les dendrites des grandes cellules multipolaires ne sortent guère du noyau. En haut, quelques dendrites d'éléments de la formation réticulée entrent dans le noyau. Chat, × 50.

en franchissant la ligne médiane; c'est la commissure protoplasmique décrite par van Gehuchten. D'autre part, des cellules de la partie dorsomédiane de la formation réticulée donnent naissance à quelques dendrites qui pénètrent dans le noyau du nerf hypoglosse et parviennent à la moitié ventrolatérale du noyau.

Les dendrites du *noyau ambigu* se ramifient aussi, en principe, à l'intérieur du noyau, mais quelques-uns en franchissent les limites dans toutes les directions. En même temps, le noyau est envahi

et couvert de dendrites provenant d'éléments réticulés voisins. De plus, les dendrites du noyau du faisceau latéral parviennent au territoire appartenant au noyau ambigu.

Au niveau du *noyau facial* et du *noyau masticateur*, presque tous les dendrites sont intrafocaux; mais il y a des exceptions, surtout dans la partie latérale où quelques cellules envoient des dendrites dans le noyau spinal du trijumeau (Fig. 3). L'extrémité marginale de ces noyaux est à peine infiltrée par les dendrites provenant de la formation réticulée voisine (Fig. 4).

*Le noyau moteur oculaire externe* a beaucoup de dendrites extrafocaux se terminant dans le noyau médian du nerf vestibulaire, le genou du facial, le faisceau longitudinal postérieur ainsi que dans la partie dorsale de la formation réticulée. A l'intérieur du noyau, on voit s'insinuer et s'entrelacer les prolongements cellulaires de la partie dorsale de la formation réticulée.

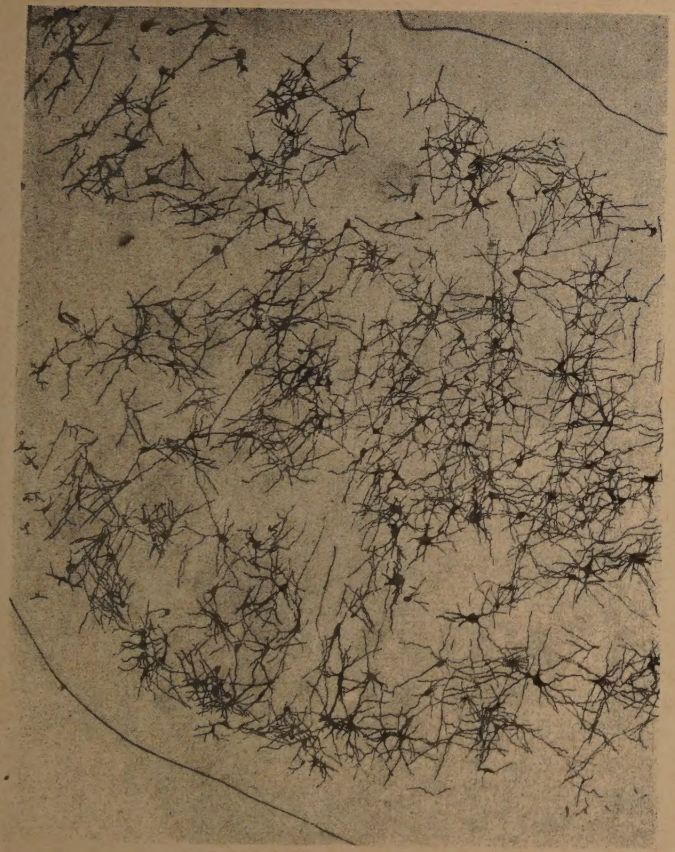
Au niveau du *noyau du nerf pathétique* et du *noyau moteur oculaire commun*, les dendrites pénètrent en avant dans le faisceau longitudinal postérieur, et, parfois même, après avoir traversé ce faisceau, dans la partie dorsomédiane de la formation réticulée. En outre, les dendrites du noyau moteur oculaire commun envahissent la substance grise centrale ainsi que le noyau oculaire opposé en formant une commissure protoplasmique.

Les éléments du *noyau médian* (*Perlia*) envoient souvent leurs dendrites dans deux directions, les uns dans un noyau latéral et les autres dans le même noyau, mais hétérolatéral.

Le *noyau d'Edinger-Westphal* échange des dendrites avec le noyau moteur oculaire commun. Ces deux noyaux sont envahis, à leur extrémité dorsale, par les dendrites des cellules de la substance grise centrale du mésencéphale.

3. *Les noyaux sensitifs du tronc de l'encéphale.* — Leurs dendrites sont presque toujours intrafocaux: il en est ainsi pour le *noyau du faisceau postérieur*, les *noyaux cochléaires*, l'*olive supérieure*, le *tubercule quadrijumeau postérieur*, les *noyaux vestibulaires*, le *noyau sensitif de l'aile grise*. Quelques *noyaux*, toutefois, tels que le *noyau spinal du trijumeau* et le *tubercule quadrijumeau antérieur*, font exception à la règle.

La plupart des dendrites du noyau spinal du trijumeau sont intrafocaux. Cependant, à tous les niveaux de la moelle allongée et de la protubérance, les éléments qui se trouvent à la périphérie



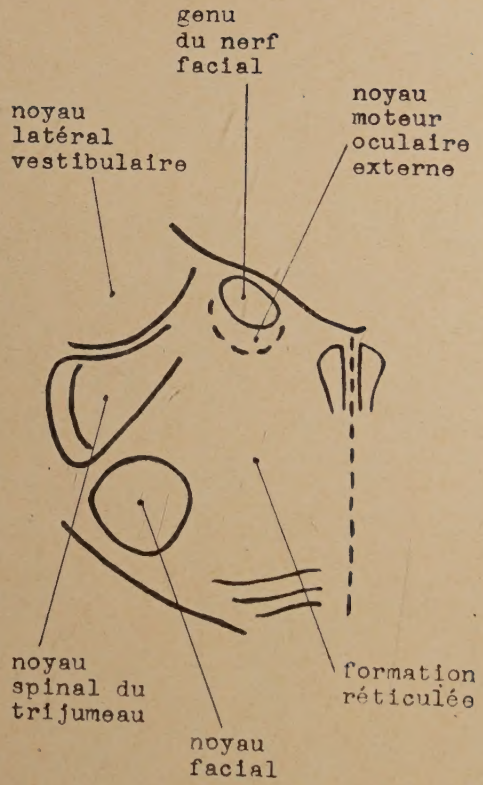



Fig. 4. – *Formation réticulée au niveau du noyau facial.*

Structure compliquée examinée au point de vue de l'arborisation dendritique. On pourrait délimiter le noyau latéral vestibulaire et le noyau facial, mais il n'est pas facile de distinguer les uns des autres le noyau moteur oculaire externe, le noyau spinal du trijumeau et la formation réticulée à cause d'échanges nombreux de dendrites. On voit que le noyau facial est envahi par des dendrites d'éléments du noyau spinal du trijumeau. Chat. Figure dessinée au moyen de l'appareil d'Abbe.

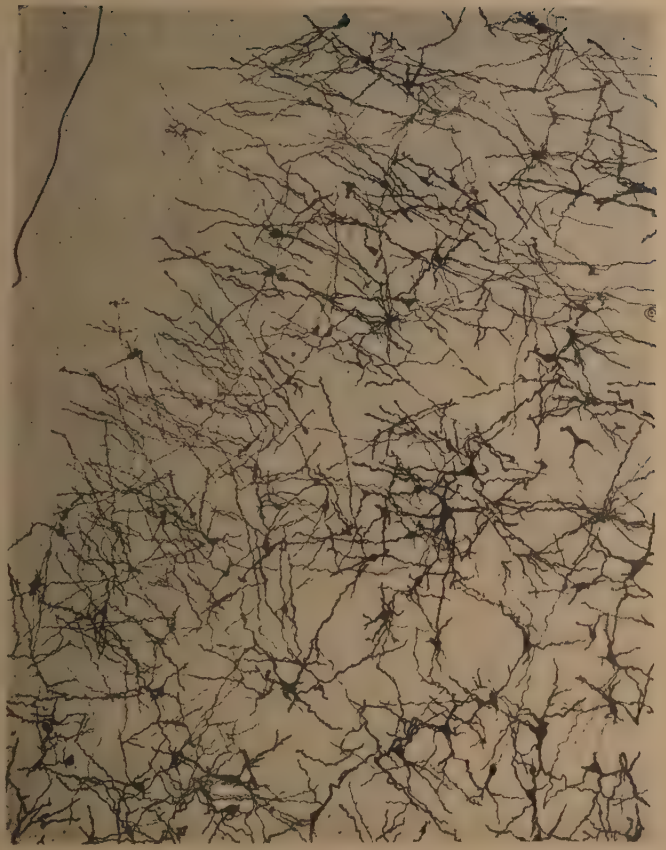
immédiate du noyau envoient leurs dendrites dans la formation réticulée avoisinante (Fig. 4). Au niveau du noyau facial, des dendrites issus de la partie externe de ce noyau pénètrent dans le noyau spinal du trijumeau; il en est de même au niveau du noyau du faisceau latéral et surtout du noyau linéaire de Cajal.

4. *La substance noire, le noyau rouge et les noyaux précérébelleux.* — Parmi les noyaux considérés comme relais de la voie extrapyramidale, la *substance noire* possède beaucoup de dendrites extrafocaux qui pénètrent dans le ruban de Reil médian, dans la formation réticulée ainsi que dans le stratum intermedium du pédoncule cérébral. Le *noyau rouge magnocellulaire* a des dendrites exclusivement intrafocaux. Il en est de même pour les noyaux cérébelleux.

Dans l'*olive bulbaire*, les *noyaux du pont*, ainsi que dans le *noyau réticulé paramédian* et le *noyau réticulé de la calotte protubérantielle*, qui sont composés essentiellement de cellules étoilées, les dendrites restent principalement dans leur noyaux respectifs ce qui n'empêche pas quelque-uns de ces noyaux d'être envahis par des dendrites provenant de la formation réticulée. Dans le noyau du faisceau latéral, les dendrites extrafocaux prédominent dans la moitié supérieure du noyau, tandis que dans la moitié inférieure les dendrites intrafocaux sont plus nombreux. Ce noyau est aussi envahi par des dendrites provenant de la formation réticulée.

5. *Le tubercule quadrijumeau antérieur, la substance grise centrale et la formation réticulée.* — Dans la préparation de Nissl ou de Weigert, on peut délimiter très nettement ces trois structures. Mais les préparations de Golgi nous montrent que la couche profonde du tubercule quadrijumeau antérieur, la moitié externe de la substance grise centrale, et même parfois sa moitié interne, échangent de nombreux dendrites. Une surface d'au moins 500 $\mu$ est ainsi couverte par les dendrites de ces deux structures. Il en est de même entre la substance grise centrale et la formation réticulée, ainsi qu'entre le tubercule quadrijumeau antérieur et la formation réticulée (Fig. 5).

De même, la division de la substance grise centrale en deux parties, extérieure et intérieure, basée sur la méthode de Nissl, devient arbitraire si l'on tient compte des dendrites. Au niveau de cette région, en effet, on constate aussi une commissure protoplasmique en avant ou en arrière de l'aqueduc mésencéphalique.



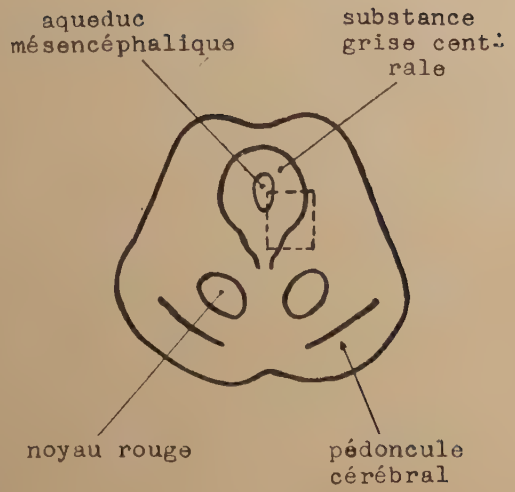



Fig. 5. – *Substance grise centrale et la formation réticulée au niveau du mésencéphale.*

A gauche, l'aqueduc mésencéphalique et, à l'extrémité droite de la figure, on trouve une cellule du noyau mésencéphalique du trijumeau qui indique la frontière entre ces deux structures. Mais il est difficile de les séparer l'une de l'autre à cause de l'échange de dendrites. Les dendrites s'arrangent d'une façon radiaire dans la

Au niveau de la formation réticulée du tronc cérébral, on distingue, du point de vue cytoarchitectonique, quelques amas cellulaires dont les dendrites s'entrelacent d'une façon compliquée, par exemple le noyau réticulé gigantocellulaire, le noyau du raphé et le noyau réticulé parvicellulaire. De plus, les cellules du raphé envoient leurs dendrites dans la formation réticulée ipsilatérale et contralatérale (Fig. 1 *d* et 4).

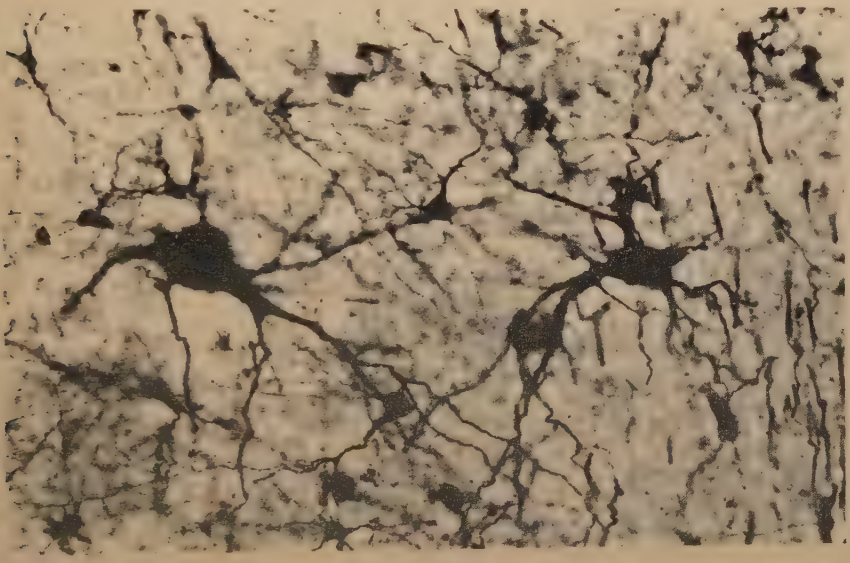


Fig. 6. — *Cellules situées près du faisceau longitudinal postérieur (moitié droite) dont l'une est contenue dans le faisceau.*

La grande cellule (gauche) envoie ses dendrites dans le faisceau. Quelques cellules névrogliques sont dispersées. Chat, × 100.

Les dendrites des cellules nerveuses s'infiltrent souvent dans la substance blanche, par exemple dans le faisceau pyramidal, le faisceau longitudinal postérieur, le ruban de Reil médian, le ruban de Reil latéral etc. (Fig. 6).

6. *Noyaux ouverts et noyaux fermés.* — Nous pourrions résumer les faits que nous venons de décrire dans les schémas suivants (Fig. 7 et 8). Quand on tient compte des dendrites extrafocaux, on peut classer les noyaux en deux groupes; ceux qui ont des dendrites extrafocaux et ceux qui n'en ont pas. Nous pourrions les ap-

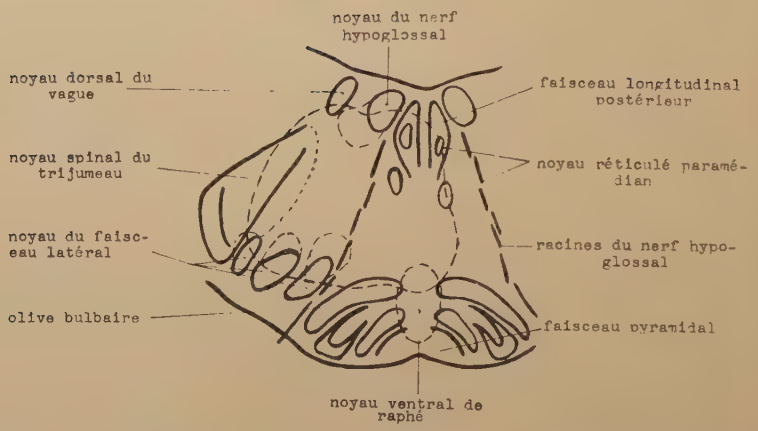



Fig. 7. – *Schéma qui montre un territoire couvert par les dendrites de la formation réticulée d'un côté.*

Ils entrent dans diverses structures avoisinantes de ce côté ainsi que dans la région pararaphale du côté opposé. En même temps, on y voit que la formation réticulée est recouverte par divers noyaux, par exemple le noyau du nerf hypoglosse, le noyau spinal du trijumeau, le noyau du faisceau latéral etc. On trouve facilement l'existance de terrains communs entre ces structures. Même dans l'intérieur de la formation réticulée, la partie médiane et la partie latérale se recouvrent réciproquement. Le faisceau pyramidal est envahi par les dendrites du noyau ventral de raphe.

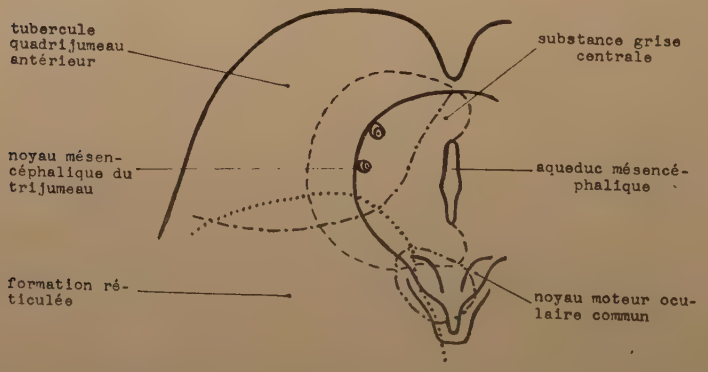



Fig. 8. – *Schéma qui montre la superposition partielle de structures mésencéphaliques.*

La substance grise centrale (——), le tubercule quadrijumeau antérieur (—.—), la formation réticulée (....) et le noyau moteur oculaire commun (—..—).

peler « noyaux ouverts » et « noyaux fermés ». Lorqu'il s'agit de « noyaux ouverts », les dendrites dépassent les limites du noyau et pénètrent dans la région avoisinante. Ainsi, le véritable territoire du noyau serait beaucoup plus grand que celui observé grâce à la méthode de Nissl ou de Weigert. La plupart des noyaux ouverts sont envahis, en revanche, par les dendrites de cellules de noyaux voisins. Deux substances grises couvrent ainsi un terrain commun et se recouvrent réciproquement par leurs dendrites; il existe alors une superposition partielle des substances grises. D'autre part, les noyaux fermés peuvent être envahis par les dendrites de noyaux ouverts voisins, bien que les premiers ne projettent pas de dendrites dans les derniers.

A ce point de vue, la plupart des noyaux sensitifs du tronc cérébral, les noyaux précérébelleux, les noyaux cérébelleux et noyau rouge magnocellulaire sont des « noyaux fermés » sauf quelques exceptions, par exemple le noyau spinal du trijumeau et les tubercules quadrijumeaux antérieurs. Le noyau du faisceau latéral, un des noyaux précérébelleux, appartient en grande partie au groupe des « noyaux fermés », et en faible partie au groupe des « noyaux ouverts ». Les noyaux moteurs du nerf crânien sont des « noyaux ouverts », à l'exception des noyaux facial et masticateur: ces derniers sont en effet presque fermés, bien qu'ils s'ouvrent dans le noyau spinal du trijumeau. La substance noire est aussi un « noyau ouvert ». La formation réticulée enfin appartient à un grand « système ouvert » qui ne contient que quelques « noyaux fermés ».

## Discussion

Beaucoup d'études cytoarchitectoniques du tronc cérébral ont été publiées jusqu'à maintenant: rappelons parmi leurs auteurs Jacobsohn (6), Winkler et Potter (21, 22), Foix et Nicolésco (3), Marburg (11), Gagel et Bodechtel (4), Stern (20), Riley (17), Krieg (8), Meessen et Olszewsky (12), Olszewsky et Baxter (13). Elles sont toutes basées sur les résultats d'observations effectuées avec la méthode de Nissl, qui permet de distinguer diverses substances grises selon les aspects du péricaryon, sa forme, son volume, sa colorabilité, sa densité etc. La délimitation des substances grises pratiquée grâce à cette méthode est assez nette. Il en est de même lorqu'on utilise la méthode de Weigert. Or, le raffinement des méthodes de stimulation électrique et d'enregistrement permet de localiser avec une

précision très grande toute région qui paraît être en rapport avec une fonction donnée. Mais les points répondant à l'excitation électrique sont indiqués, dans la plupart des travaux neurophysiologiques, sur des diagrammes simplifiés de coupes cérébrales, colorées uniquement par la méthode de Nissl ou bien de Weigert.

Jusqu'à présent, on n'a presque pas tenu compte de l'arborisation dendritique dans la démarcation des différentes parties de la substance grise. Cette démarcation n'est certainement pas aussi facile avec la méthode de Golgi que sur les préparations de Nissl ou de Weigert. A cet égard, les notions de « noyau fermé » et de « noyau ouvert » ainsi que de « superposition partielle des substances grises », que nous avons introduites, nous semblent très importantes au point de vue morphologique autant que physiologique.

Lorsqu'il s'agit d'un « noyau fermé », on peut indiquer la localisation d'une lésion de façon précise avec les préparations de Nissl ou de Weigert. Pour le « noyau ouvert », il n'en est pas de même. Si la lésion est localisée à l'intérieur du noyau, il n'y a pas de problème. Mais si la lésion siège dans un territoire couvert par des dendrites extrafocaux, sans aucune trace de lésion du noyau lui-même, ..i qui travaille avec les méthodes de Nissl ou de Weigert peut arriver à la conclusion erronée que les neurones du noyau n'ont pas été atteints.

Il en est de même lors de stimulation. Comme à un point donné se concentrent beaucoup de dendrites d'éléments proches ou éloignés, la stimulation électrique peut intéresser un nombre de neurones beaucoup plus élevé qu'on pourrait le croire en étudiant des coupes au Nissl ou au Weigert. La stimulation d'un point commun entre deux noyaux ouverts résultera donc des effets de l'excitation simultanée de tous les deux. Il s'en suit qu'il ne faut pas considérer seulement la diffusion physique du stimulus, qui peut varier avec le type d'électrode employé et l'intensité du courant, mais aussi les différences morphologiques, qui peuvent modifier profondément le nombre et la qualité des neurones excités.

Brodal (1) a montré que la formation réticulée n'est pas uniforme dans son organisation morphologique. Au niveau de la moelle allongée, la région médiane envoie une grande quantité de fibres en direction rostrale ou caudale: elle pourrait être considérée d'après Brodal comme partie « effectrice », tandis que la région latérale de la formation réticulée serait la partie « sensitive » ou « associative ». Toutefois la limite entre ces différentes régions n'est pas nécessaire-

ment nette et les données anatomiques nous montrent qu'il y a beaucoup d'interactions et de corrélations possibles entre elles. En effet, les dendrites de ces deux régions s'entrelacent d'une façon si compliquée qu'il est vraiment difficile de les délimiter avec netteté: ces deux régions se superposent donc partiellement. Dans la région médiane on distingue le noyau gigantocellulaire, le noyau paramédian, le noyau ventral du raphé etc., mais en réalité ces structures se superposent ça et là et leur limites sont presque impossible à préciser. Le calcul des Scheibel (18) nous montre que, dans le territoire occupé par le péricaryon et les dendrites d'un élément du noyau gigantocellulaire, il existe environ 4125 cellules nerveuses.

D'après les résultats de stimulations de la moelle allongée par Pitts (14), Pitts, Magoun et Ranson (15) et d'autres, les parties médiane, rostrale et dorsale du noyau réticulé gigantocellulaire constitueraient le centre expiratoire tandis que la partie parvicellulaire serait considérée comme le centre principal de l'inhibition de l'inspiration. La limite entre ces deux structures n'est pas toujours nette. De plus, il est à noter que, comme le montrent les figures de Pitts (15), des points expiratoires et inspiratoires se trouvent dans le noyau du nerf hypoglosse, dans nucleus prepositus hypoglossi, dans le noyaux spinal du trijumeau et du faisceau latéral, dans l'olive bulbaire etc. Ces structures, sauf l'olive bulbaire, sont des noyaux ouverts; elles sont pénétrées en outre par des dendrites d'éléments de la formation réticulée. L'olive bulbaire elle aussi est envahie en certains endroits par des dendrites d'éléments réticulés, ou appartenant aux noyaux du faisceau latéral ou du raphé. Les effets de la stimulation de ces structures seront donc nécessairement compliqués par ceux dûs à l'excitation de la formation réticulée elle-même. Mais il y a plus: la stimulation unilatérale de la région située près du raphé entraînera l'excitation non seulement d'éléments homolatéraux, mais aussi d'éléments hétérolatéraux; et cela d'une façon directe, par l'intermédiaire des commissures dendritiques.

Les données de l'anatomie expérimentale peuvent être sérieusement affectées, elles aussi, par ces considérations. La nouvelle méthode de Rasmussen (16) nous montre que le péricaryon ainsi que les dendrites sont couverts par des boutons terminaux très nombreux et les méthodes de Marchi, Glees ou Nauta nous permettent de suivre le trajet d'une voie jusqu'à sa terminaison, en profitant de la gaine myélinique ou du cylindraxe en voie de dégénérescence. La présence d'une telle dégénérescence dans un noyau

donné ne peut pas nous permettre de conclure que cette voie s'y termine; en effet s'il s'agit d'un noyau ou d'un territoire commun entre deux noyaux ouverts, il est possible que la terminaison de cette voie se mette en contact non seulement avec les cellules de ce noyau, d'une façon axo-somatique, mais aussi avec les dendrites d'éléments avoisinants qui envahissent l'intérieur du noyau, et celà d'une façon axo-dendritique. Quant à la voie qui se termine près du raphé, il est également possible qu'elle soit en contact avec des éléments du côté opposé par l'intermédiaire de commissures dendritiques.

Rappelons enfin que même la substance blanche compacte contient beaucoup de dendrites issus des cellules nerveuses voisines: sa stimulation produira donc non seulement une excitation des fibres du faisceau mais aussi celles des cellules nerveuses voisines, effet analogue à celui de la stimulation directe de la substance grise.

Enfin, il faut considérer les différentes modalités de l'arborisation dendritique. La comparaison des données des quelques cas que nous avons examinés nous permet de constater que l'étendue de l'arborisation ainsi que le degré de la superposition partielle des substances grises sont assez variables suivant les cas. Les résultats d'expérimentations physiologiques peuvent être tout aussi variables, bien que l'on stimule le même point dans les mêmes conditions; ce fait pourrait être dû à cette différence morphologique individuelle d'ordre microscopique. Nous sommes donc tout à fait d'accord avec Lorente de Nó (10), quand il écrit au sujet de la cytoarchitecture de la corne d'Ammon: « The authors who are not accustomed to work with the silver ou mercury chromate methods of Golgi, will perhaps think that the foregoing division of the Ammonshorn celles is artificial and too extreme. The opposite would be true. The above classification is still too incomplete. In spite of the efforts of Golgi, Sala, Schaffer, Lugaro, Kölliker, and chiefly of Cajal, and of my modest work, the present description of the Ammonshorn can not be considered more than a preliminary communication. Every one of the cell types mentioned here is perfectly justified. When two cells have similar axonal apparatus, but dendrites which are distributed differently, they belong to two different types, because they receive different impulses. When two cells have similar dendrites, but different ramified axons, they again belong to different types because they transmit their impulse in a different manner. All of the types described here have different dendrites and different axons ». Il en est exactement de même au niveau du tronc cérébral.

## Résumé

Nous basant sur les résultats d'observations faites grâce à la méthode de Golgi sur le tronc cérébral, surtout sur la formation réticulée de l'enfant et du chat et du lapin nouveaux-nés et en les comparant avec les données cytoarchitectoniques obtenues grâce à la méthode de Nissl ou de Weigert, on voit que les noyaux peuvent être classés en deux groupes: « noyaux fermés », qui n'ont pas de dendrites extrafocaux, et « noyaux ouverts » qui en ont beaucoup. Il se peut parfois que même le noyau fermé soit envahi par des dendrites d'éléments avoisinants. Lorqu'il s'agit d'un noyau ouvert, l'extension du territoire qui est couvert par la totalité de dendrites extrafocaux et intrafocaux est plus grande que dans les préparations au Nissl ou au Weigert. On trouve un échange intense de dendrites entre deux noyaux ouverts avoisinants; ils s'étendent donc sur un terrain commun, et sont partiellement superposés. Il est ainsi possible d'obtenir, par la stimulation de l'un de ces deux noyaux, la réponse simultanée des deux. L'effet de stimulation serait donc beaucoup plus compliqué que l'on s'imagine. De plus, les cellules qui se trouvent près de la ligne médiane envoient des dendrites vers l'autre côté; il en résulte que la stimulation ou la destruction d'un côté provoque l'excitation immédiate du côté opposé par l'intermédiaire de la commissure dendritique. Les notions de « noyau fermé » et « noyau ouvert » ainsi que de « superposition partielle de substances grises » seraient donc importantes non seulement au point de vue morphologique mais aussi au point de vue physiologique.

A ce point de vue, la plupart des noyaux moteurs des nerfs crâniens sont des « noyaux ouverts », à l'exception du noyau facial et du noyau masticateur, tandis que les noyaux sensitifs appartiennent aux « noyaux fermés », sauf le noyau spinal du trijumeau. Le tubercule quadrijmeau antérieur est un « noyau ouvert », tandis que le tubercule quadrijumeau postérieur appartient aux « noyaux fermés ». Les noyaux précérébelleux et les noyaux cérébelleux sont aussi des « noyaux fermés ». Mais le noyau du faisceau latéral, un des précérébelleux, appartient en grande partie aux « noyaux fermés » et pour une petite partie seulement aux « noyaux ouverts ». Parmi les relais de la voie extrapyramidale, la substance noire est un « noyau ouvert ». Enfin, la formation réticulée est un vaste « système ouvert » contenant seulement quelques « noyaux fermés ».

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# ACTION OF 5-HYDROXYTRYPTAMINE AND OF 5-HYDROXYTRYPTOPHAN ON THE CORTICAL ELECTRICAL ACTIVITY OF THE MIDPONTINE PRETRIGEMINAL PREPARATION OF THE CAT WITH AND WITHOUT MESENCEPHALIC HEMISECTION [1]

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## Introduction

Prompted by the hypothesis that the 5-hydroxytryptamine (5HT) present in the brain plays some part in the activity of the central nervous system, there have been several investigations of the EEG effects of the anime. Gangloff and Monnier (9) observed, in the rabbit with intact neuraxis, after intravenous injection of 0.1-1 mg/kg of 5HT, a short period of electroencephalic activation, followed by a second phase of greater synchronization and then a third phase of desynchronization. These results were confirmed by Rothballer (18) in the curarized cat with intact neuraxis or with lesions in the mesencephalic reticular substance. One of us (12) has described a clear arousal reaction following the injection of minute amounts of amine into the carotid or vertebral circulations in the *encéphale isolé* preparation of the cat with vagi and trigeminal nerves cut and with the carotid sinuses denervated.

The demonstration that the 5HT carried by the blood modifies the electrical activity of the cerebral cortex can be held to support

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[1] A preliminary note has been published in *Experientia*, *16*: 213-214, 1960. English translation by T. D. M. Roberts and J. D. Christie.

the hypothesis that the amine acts directly on the cerebral neurones, only if two presuppositions turn out to be true: 1) that the EEG effects do not depend on the changes in the cerebral circulation produced by the substance, or on stimulation of 5HT-sensitive receptors at some possible intracerebral site, and 2) that the 5HT circulating in the blood crosses the blood-brain barrier and can reach the nerve cells. Some doubt is cast on the validity of the first of these suppositions by the results of Crepax and Infantellina (7). They observed, in the cat with intact neuraxis or *cerveau isolé*, a flattening of the EEG trace after the intracarotid injection of 200-750 $\mu$g of 5HT; this effect the authors attribute to changes in the cerebral circulation, even in the absence of changes in the systemic blood-pressure. Recent biochemical findings (19) make one regard the second supposition as improbable, and at the same time suggest an other way of approaching the problem of the physiological action of the cerebral 5HT, namely by the use of 5-hydroxytryptophan (5HTP), the amino-acid precursor of 5HT, which certainly penetrates into the central nervous system and is transformed locally into amine. It is indeed reasonable to suppose that the excess of amine produced by the 5HTP gives rise to effects which can be considered qualitatively identical with those developed by the 5HT normally synthesized in the nervous tissue.

The aim of the experiments reported here was to compare in the cat the effects of 5HT and of 5HTP, injected directly into the cerebral circulation, on two different electroencephalographic patterns: on the activated pattern of the midpontine pretrigeminal preparation and on the synchronized pattern of the same preparation after mesencephalic hemisection. The effects of the two substances on the cerebral circulation were recorded at the same time.

The EEG effects of intravenous injection of 5HTP in the rabbit had already been described by Monnier and Tissot (16) and by Costa and Rinaldi (6). Monnier and Tissot found a synchronizing effect at low doses (10-20 mg/kg) and an activating effect at high doses (30 mg/kg and more i. v.) of the amino-acid; the activating effect remained after a midbrain section which disconnected the brain from all the afferent pathways except the optic and olfactory pathways. Costa and Rinaldi observed, after 75 mg/kg i. v., a reduction in the voltage of the cortical electrical activity, preceded by a phase with slow waves of very much simplified shape.

## Methods

Our experiments were carried out on 35 adult cats. The midpontine pretrigeminal section was performed, with the animal under ether narcosis, using a spatula sliding in a plane inclined at 45° and oriented stereotaxically. Occasionally, to avoid bulbar compression by the accumulation of blood in the IVth ventricle, the cerebellum was partly removed by suction. In agreement with Batini, Moruzzi, Palestini, Rossi and Zanchetti (2) the EEG pattern in this preparation was characterized for long periods of time by fast, low-voltage rhythms, indicative of a state of wakefulness. Respiration and blood-pressure (120-140 mm Hg) were normal.

In one group of experiments, after the midpontine section, we carried out a hemisection of the mesencephalic reticular substance by the technique described by Cordeau and Mancia (5). In agreement with these authors, the EEG record after the section was markedly asymmetrical: the hemisphere above the mesencephalic lesion showed synchronization, while the hemisphere of the opposite side was desynchronized. This asymmetry could be replaced, for long periods, by bilateral synchronization or, rarely in our experimental conditions, by bilateral desynchronization. The mesencephalic reticular formation of the side opposite to that of the hemisection was stimulated with bipolar concentric electrodes, oriented stereotaxically. The localization of the sections and of the stimulating electrode was checked histologically. In both of the preparations, the occlusion of one common carotid artery did not produce any depression of the cortical electric activity of the corresponding hemisphere; only exceptionally did it produce a transitory arterial hypertension, while in every case it produced a lasting increase in the flow of blood in the common carotid artery of the opposite side.

The injection was made into the carotid circulation. The substances were injected through a polythene cannula fixed into the proximal end of the lingual artery: the injected liquid thus reached the lumen of the external carotid artery and was carried to the cerebral circulation by the normal movement of the blood. In most of the experiments, to make sure that the substances would flow to the opposite side of the brain, the common carotid artery of the opposite side was occluded a few minutes before the injection. Two methodological checks were invariably carried out: *a*) at the beginning of the experiment, before the mesencephalic hemisection, minute amounts of Thiopental (0.5-1 mg) were injected into the carotid artery: the EEG synchronization was restricted to the side of the injection when the carotid artery of the other side was open; whereas it spread to both hemispheres when the carotid artery of the opposite side was closed; *b*) at the end of the experiment, after exposure of the cerebral hemispheres, 0.2 ml of a solution of sodium fluorescein 0,1% was injected by the same route and the distribution of the fluorescein to the two hemispheres was observed under ultraviolet light: it was restricted to the side of the injection when the carotid artery of the opposite side was open; whereas it spread to both hemispheres when the carotid artery was closed. For injection of the substances into the general circulation and for the infusion of adrenaline a saphenous vein was used.

The recording of the carotid blood flow made it possible to follow in each experiment the action of 5HTP and of 5HT on the cerebral circulation. The changes in blood-flow in the common carotid artery were recorded on the side of the injection by the method of Rein's Thermostromuhr. The changes in flow produced by the two substances were compared to those produced by occluding the common carotid artery of the opposite side and, in some experiments, to those produced by adrenaline and by acetylcholine

injected by the same route. The arterial pressure was recorded from the femoral artery with an Elema electromanometer connected to a mirror galvanometer writing on a photokymograph. The recordings of EEG, of pressure, and of blood-flow were carried out simultaneously.

The cortical electrical activity was recorded with unipolar screw electrodes fixed in the skull at the levels of the frontal, parietal, and occipital areas of both sides. A 6-pen Schwarzer electroencephalograph was used.

The 5HT (double sulphate of 5HT and creatinine), the 5HTP (racemic form), the adrenaline (*l*-adrenaline hydrochloride), the acetylcholine (acetylcholine bromide), and the Thiopental Sodium were dissolved in Tyrode at the concentrations respectively of $2 \times 10^{-5}$, $1 \times 10^{-2}$, $1 \times 10^{-5}$, $1 \times 10^{-5}$, $5 \times 10^{-3}$, and were injected into the carotid artery as nearly as possible at a constant speed. The rate of intravenous infusion of adrenaline was 3-5 $\mu$g/min. The values by weight of the 5HT refer to the free base.

## Results

### I. – *Effects of 5HT and of 5HTP on the EEG, on the arterial pressure, and on the carotid blood-flow in the midpontine pretrigeminal preparation.*

1. *Effects of 5HT.* — 5HT (2-80 $\mu$g) injected slowly (1-3 $\mu$g/sec) into the carotid artery, the artery of the opposite side being occluded, never produced any change in the electroencephalographic record, at any rate up to 15-20 min from the end of the injection (Fig. 1 *B*). Note that only a synchronizing effect would have been able to show itself against the steadily activated EEG pattern of this preparation.

The circulatory effects of the 5HT were variable. Doses less than 10 $\mu$g were nearly always without any effect on the systemic pressure, and only occasionally gave a small and transitory hypotension. Higher doses ($> 40\,\mu$g) regularly produced a prolonged slow decline in pressure, to a minimum of 90 mm Hg. The lower doses either did not change the carotid blood-flow even when the arterial pressure fell, or produced a slight increase in flow; only exceptionally did they produce a reduction in flow. The higher doses frequently produced a reduction in flow coinciding with the greatest hypotension; in some experiments, however, 5HT even at a dose of 80 $\mu$g did not alter the carotid blood-flow. The amplitude of the changes in flow in the same experiment were variable, not proportional to the dose of 5HT, and in every case less than 1/4 of the change produced by closing the carotid artery. In the same preparation, acetylcholine (0.5-1.5 $\mu$g) regularly produced an in-

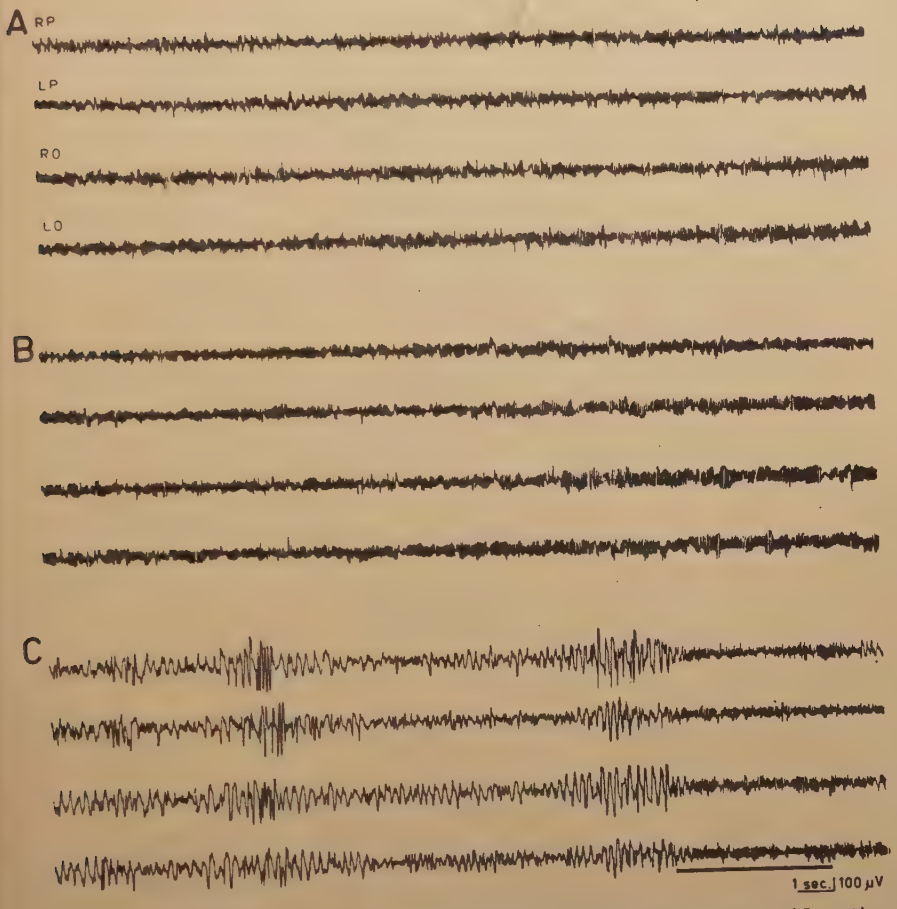


Fig. 1. – *Effects of 5HT and of 5HTP on the EEG of the midpontine pretrigeminal preparation.*

*A*: the activated record of the midpontine pretrigeminal preparation; *B*: 4 min after the injection of 80 µg of 5HT into the right carotid artery, the artery of the opposite side being closed: EEG unchanged; *C*: 5 min after the injection of 15 mg of 5HTP: the EEG synchronization is clearly seen; olfactory stimulation (indicated by the signal line) produces a definite arousal reaction. In this and subsequent figures are shown records obtained with unipolar leads from the parietal (P) and occipital (O) areas of the right (R) and left (L) sides, as indicated. This and the following illustrations have been retouched.

crease and adrenaline (1-3 µg) a decrease in flow. The changes produced by the higher doses were often a little smaller than those produced by closing the carotid artery and were always much greater than the largest observed after 5HT.

2. *Effects of 5HTP.* — 5HTP (8-20 mg) injected slowly into the carotid artery of one side, the contralateral artery being closed, changed the desynchronized EEG pattern of the midpontine pretrigeminal preparation to a pattern of marked synchronization (Fig. 1 *C*), entirely similar to that produced by Thiopental injected by the same route. The synchronizing effect began within 1-3 min from the end of the injection, occasionally during the injection, and lasted for more than 30-45 min. In most of the preparations, visual and olfactory stimulations gave a clear arousal reaction. At higher doses, up to 40-60 mg, the slowing of the cortical electrical activity was more marked and the arousal reaction to visual and olfactory stimulation was at times abolished. The circulatory effects of 5HTP were usually slight: only in some experiments did the systemic pressure fall to 100-110 mm Hg; the carotid blood-flow, unchanged in some cases, behaved differently in others, increasing and decreasing at times in a transitory manner. It was not possible to establish a relation between the behaviour of the systemic pressure and that of the carotid blood-flow, whereas it was quite clear that the EEG changes were totally independent of either the generalized circulatory changes or of those localized in the carotid region: indeed, in most cases, the synchronization of the cortical electrical activity was not accompanied by any circulatory change, or still persisted long after the carotid blood-flow and the systemic pressure had returned to their original values.

The synchronizing effect of 5HTP could have been due: *a*) to products of the extracerebral metabolism of the amino-acid carried by recirculation into contact with the C. N. S., or *b*) to products of the intracerebral metabolism of the amino-acid, or finally *c*) to the amino-acid itself. To clear up at least the first point, we injected 5HTP into the carotid artery of one side, leaving open the carotid artery of the opposite side; in this way it was possible to obtain, during the injection, a difference in concentration of 5HTP in the blood, as between the circulatory areas of the two carotid arteries. The concentration in the blood in the half of the brain on the side of the injection was kept up by the infusion of amino-acid, while the concentration in the blood in the opposite half of the brain was only raised by that amount of amino-acid which, after escaping at its first passage through the brain, returned symmetrically to the two carotid arteries. In contrast, the products of the extracerebral metabolism of the 5HTP always arrived in equal quantities at

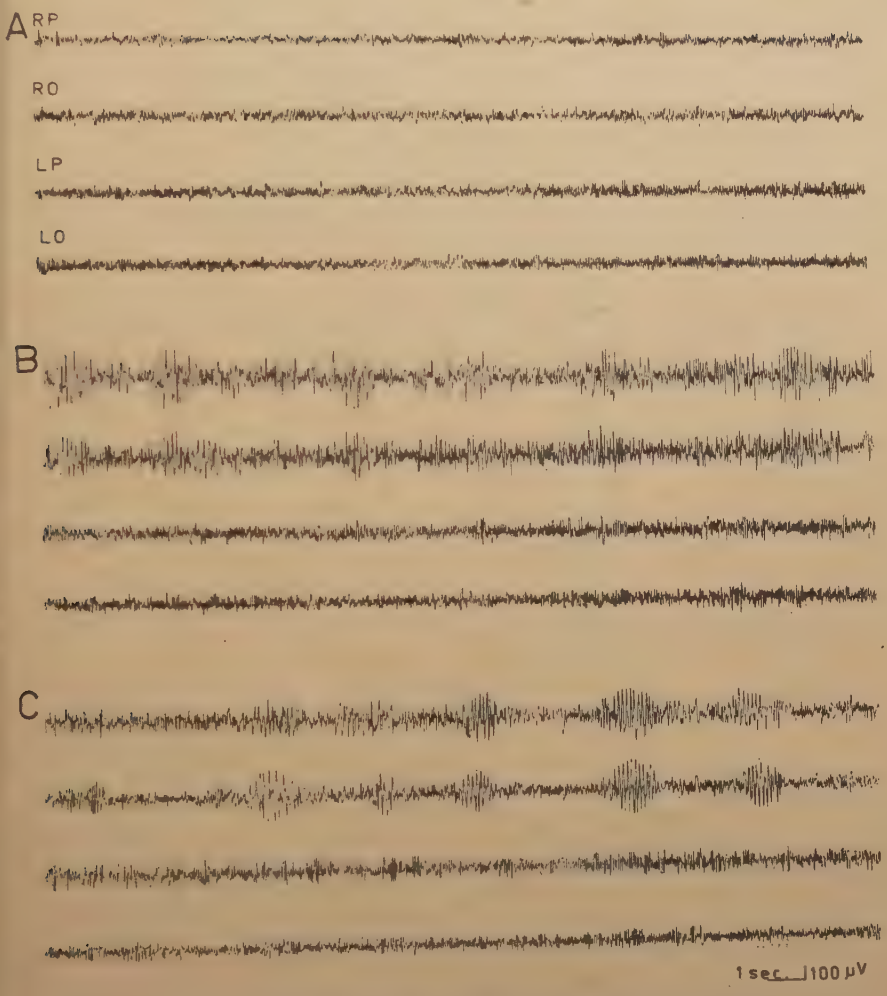


Fig. 2. – *EEG asymmetry produced by Thiopental and by 5HTP injected into the carotid artery of one side, the contralateral artery being open.*

*A*: symmetrically activated EEG record; *B*: immediately after the injection into the right carotid artery of 1 mg of Thiopental; *C*: 1 hr after *B* and 3 min after the injection into the right carotid artery of 20 mg of 5HTP. In *B* and *C* the right hemisphere is clearly synchronized while the left hemisphere is desynchronized as in *A*.

the two carotids, through the recirculation. Any difference in the EEG between the two hemispheres would thus have to be attributed to the difference between the concentrations of 5HTP in the blood at the level of the two halves of the brain during the injection.

5HTP (8-20 mg), injected in these conditions, produced a clear asymmetry in the EEG: the hemisphere on the side of the injection showed a greater synchronization than the hemisphere of the opposite side (Fig. 2 *C*). The asymmetry tended to disappear 10-15 minutes after the injection, but an awakening stimulation could show it up afresh: the arousal reaction was more persistent in the hemisphere of the side opposite to the injection. At lower doses the 5HTP (4-8 mg) produced a latent asymmetry, only shown up by the intravenous injection of small doses of Thiopental (2-3 mg/kg), which produced a more marked EEG synchronization on the side of the injection of 5HTP.

Summarizing: in the midpontine pretrigeminal preparation, 5HTP produces a clear picture of EEG synchronization, while 5HT is entirely inactive. The effect of 5HTP does not depend on bloodborne products of extracerebral metabolism of the amino-acid.

II. – *Effects of 5HT and of 5HTP on the EEG, on the arterial pressure, and on the carotid blood-flow in the midpontine pretrigeminal preparation with mesencephalic hemisection.*

1. *Effects of 5HT.* — 5HT (1-4 $\mu$g) injected into the carotid artery of one side, the contralateral artery being closed, frequently produced an activation of the symmetrically or asymmetrically synchronized EEG record of this preparation (Fig. 3 *B*). The effect began during or at the end of the injection and lasted for some tens of seconds. The cerebral and systemic vascular effects of the 5HT were the same as those already described in paragraph I, 1. In some experiments the preparation was paralysed with Flaxedil and maintained with artificial respiration: 5HT then gave more often a decrease in carotid blood-flow. In these cases it might be suspected that the EEG arousal was the consequence of a slight asphyxia of the cerebral neurones produced by the reduction in blood-flow. We have therefore tried to discover *a*) whether the intracarotid injection of adrenaline, which produced a marked reduction in blood-flow, reproduced the same EEG effect as 5HT, and *b*) whether, using an intravenous infusion of adrenaline to modify the effects of 5HT on the cerebral circulation, one could abolish the EEG effect of the amine. Not only did the reduction in blood-flow produced

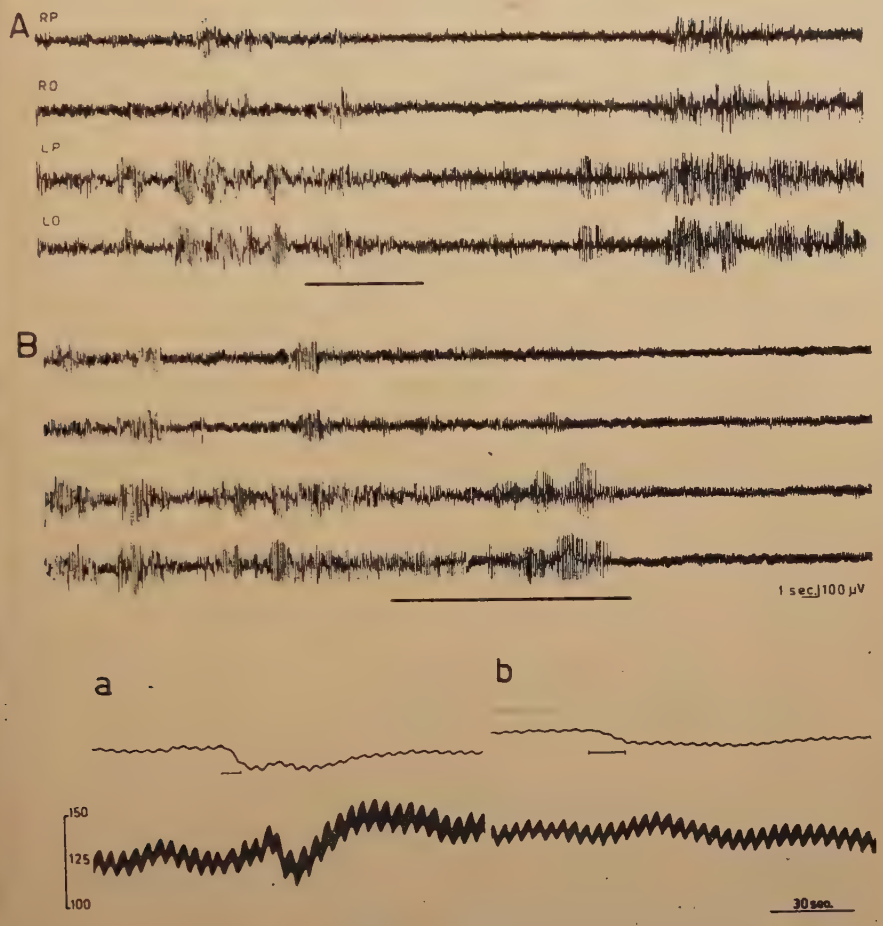


Fig. 3. – *Effects of adrenaline and of 5HT on the EEG, on the carotid blood-flow, and on the arterial pressure, in the midpontine pretrigeminal preparation with mesencephalic hemisection.*

Curarized preparation; mesencephalic hemisection on the left side; injection and recording of blood-flow on the right side; left carotid closed. *A* and *B*: EEG traces. *a* and *b*: records of carotid blood-flow (upper trace) and systemic blood pressure (lower trace). Drugs were injected at the times indicated by the signal lines. *A* corresponds with *a* and *B* with *b*. *A*: the injection of 2 µg of adrenaline (indicated) does not alter the synchronized EEG, while (in *a*) it produces a sudden reduction in blood-flow (upper trace) followed by a rise in systemic pressure; *B*: the injection of 4 µg of 5HT (indicated) activates the EEG, produces (in *b*) a smaller reduction in blood-flow than in *a*, and does not alter the systemic pressure.

by adrenaline (1-3 µg) fail to produce EEG arousal (Fig. 3 *A*), but it was also possible, with the intravenous infusion of adrenaline (3-5 µg/min), to abolish or reverse the reduction in blood-flow

without abolishing the arousal reaction produced by 5HT. These findings do not necessarily exclude the possibility that changes in the cerebral circulation contribute to the production of the EEG arousal caused by 5HT. In other cases, however, the same effect occurred in spite of the absence of any change in the carotid blood-flow. Doubtless some objections might be raised to the method we have used for studying the cerebral circulation. The amount of blood which passes through the section of common carotid in unit time depends not only on the systemic arterial pressure, but equally on the overall peripheral resistance offered by the two areas of distribution of the common carotid artery: the extracranial area and the intracranial area. Suppose for the moment that the 5HT has opposite actions on these two areas: this would imply that its effect, as seen at the level of the common carotid artery, would be less than or even of opposite sign to the true effect on one of these two regions. It is, however, very hard to believe that, in those cases in which the EEG effect of 5HT is not accompanied by any change in carotid blood-flow, there is such an exact compensation between the vascular effects in the two areas of distribution of the common carotid artery, that there is no visible effect at the level of the carotid. One can, nevertheless, imagine vascular changes in the intracranial circulation of so restricted a nature as to be below the limits of sensitivity of the method we used.

2. *Effects of 5HTP.* — In the midpontine pretrigeminal preparation with mesencephalic hemisection, 5HTP (2-20 mg) never produced an EEG arousal; on the contrary, in doses above 5mg it induced a bilateral state of EEG sleep, and often abolished the arousal reaction to olfactory stimulation. The threshold at which electrical stimulation of the mesencephalic reticular substance just produces EEG activation in the hemisphere above the midbrain lesion, remained unchanged by these doses of 5HTP, while it was raised by 2 or 3 volts with higher doses (30-50 mg); blocking of the arousal reaction to electrical stimulation of the mesencephalic reticular substance was never observed.

Summarizing: in the midpontine pretrigeminal preparation with mesencephalic hemisection, 5HT produces a desynchronization of the EEG record, probably not bound up with changes in the cerebral circulation. In the same preparation, 5HTP produces an accentuation of the sleep type of EEG pattern.

III. – *EEG effects of 5HT on the midpontine pretrigeminal preparation after sensory de-afferentation.*

One might suppose that the desynchronizing effect of 5HT described in paragraph II, 1 could arise from stimulation of the visual or olfactory receptors, the only receptors known to be still connected with the brain after the pontine section. We have accordingly carried out some control experiments in the midpontine pretrigeminal preparation after section of the optic nerves and removal of the olfactory bulbs. Immediately after the de-afferentation, the EEG picture in this preparation, in agreement with Batini, Palestini, Rossi and Zanchetti (3), was characterized by slow, high-voltage waves, usually associated with sleep, and 5HT (1-4 $\mu$g) gave a clear arousal reaction, as described in paragraph II, 1.

## Discussion

The object of the experiments reported here was to compare the EEG effects of 5HT with those of 5HTP, the amino-acid precursor of 5HT. The results set out above lead one to suppose that the amino-acid and the corresponding amine have different effects. It seems to us advisable to discuss the actions of 5HT and of 5HTP separately.

The only EEG effect observed with 5HT was the desynchronization of the synchronized EEG pattern in the midpontine pretrigeminal preparation after mesencephalic hemisection or sensory de-afferentation; it was not possible to see any synchronizing effect of the amine on the activated record in the same preparation before the mesencephalic hemisection or the sensory de-afferentation. As the pretrigeminal section interrupted all the sensory pathways to the brain with the exception of the optic and olfactory pathways, which were, however, cut in control experiments, one can exclude the possibility that the arousing effect of 5HT is a consequence of the stimulation of peripheral receptors. It is necessary, therefore, to think in terms either of a direct effect of the amine on the cerebral neurones, or of an indirect effect, caused either by metabolic changes consequent upon vasomotor alterations in the cerebral circulation or by the stimulation of 5HT-sensitive chemoreceptors at some intracranial site.

On the basis of the results set out above, one can exclude the possibility that gross changes in the cerebral circulation are responsible for the EEG effects of 5HT, but it is still possible to imagine that changes localized to certain vascular regions, and not manifesting themselves as changes in the carotid blood-flow, could produce metabolic alterations in restricted regions of the C. N. S. with repercussions on the electrical activity of the cerebral cortex.

Amin, Crawford and Gaddum (1) have examined the second possibility, namely that 5HT can act indirectly on the nerve-cells by way of the stimulation of 5HT-sensitive receptors, situated more peripherally, in the vascular sense, than the carotid glomus, in point of fact in the neuroglia. This hypothesis is based on two findings: *a*) the neuroglia can collect the 5HT circulating in the blood: indeed the « area postrema », which does not contain true nervous tissue but only neuroglia, is rich in 5HT, presumably not produced locally, – because this area has no 5HTP-decarboxylase (8) – but picked up from the blood; *b*) neuroglial elements cultivated *in vitro* by Benitez, Murray and Wolley (4) and Miura *et al.* (15) contract strongly on the addition of 5HT. That is to say: the 5HT in the blood can be picked up by, and can act on, elements of the neuroglia; it remains to be seen whether the neuroglia can interfere in the activity of the nervous elements.

If these two considerations make it difficult to attribute the EEG effects which we have observed to the direct action of the amine on the cerebral neurones, there is yet another set of objections which could be raised against a similar interpretation of the results reported by other authors. Gangloff and Monnier (9) in the rabbit, and Rothballer (18) in the cat, obtained, after a brief phase of EEG activation, a phase of synchronization followed by one of desynchronization. It is known [1] that 5HT stimulates the chemoreceptors and pressoreceptors of the carotid sinus, the pulmonary vagal receptors, and the intracardiac receptors responsible for Bezold-Jarisch reflex, and produces, among other things, an increase in intestinal motility and contraction of the bradder. In the experiments of these authors, the discharge of nervous impulses arising from all the peripheral receptors, excited directly or

[1] For a review of the peripheral effects of 5HT see Page (17).

indirectly by 5HT, were free to reach the cerebral structures and to interfere with the supposed direct action of 5HT on the cerebral neurones in such a way as to make it impossible to recognize what part of the EEG changes was attributable to a possible central action of 5HT. The same considerations weaken the results obtained by Crepax and Infantellina (7) on the curarized cat with intact neuraxis. The same authors, however, in the *cerveau isolé* preparation (in which the central action of the amine could be inventigated independently from perypheral effects) never observed any arousal reaction. They tested for an effect of 5HT on the pattern of cortical synchronization produced by a complete midbrain section, while in our experiments the cortical synchronization in the midpontine pretrigeminal preparation was obtained either by mesencephalic hemisection or by sensory de-afferentation. As our results differ from those of Crepax and Infantellina it is possible that the portion of reticular substance between the midbrain section and the pretrigeminal section is essential for the appearance of the arousing effect of 5HT.

5HTP produced EEG synchronization in the midpontine pretrigeminal preparation, where 5HT was apparently inactive, and increased the degree of synchronization in the same preparation after mesencephalic hemisection, where 5HT had a desynchronizing effect. The fact that the EEG changes are limited, strictly or predominantly, to the hemisphere corresponding to the side of the injection, is sufficient evidence for the assertion that neither the products of the extracerebral metabolism of the amino-acid nor substances liberated by the amino-acid and circulating in the blood were responsible for the effects observed. On the other hand, it is clear that the peripheral autonomic effects of the amino-acid or of the corresponding amine circulating in excess in the blood could not have interfered by nervous pathways with the brain, as this was isolated by the pretrigeminal section.

Some of the products of the intracerebral metabolism of the amino-acid are known: 5HT and 5-hydroxy-indolyl-acetic acid [1]. It might be supposed that either of these substances or the amino-

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[1] Other products of the metabolism of 5HTP, or chemically related substances, have recently been described as present in places other than the brain: N-acetyl-5HT and the *o*-glucuronide of 5HT, found in the urine of animals treated with 5HT (14) and 5-methoxy-N-acetyl-tryptamine (10).

acid itself could give rise to the effect described. Although no crucial evidence has been obtained, there are some findings which support the idea that 5HT is the substance responsible for the central effects of 5HTP: *a*) iproniazid, a powerful inhibitor of amine-oxidase, enhances in the rabbit the neurological effects of 5HTP and at the same time increases the amount of 5HT in the brain (19); *b*) the same enzyme-blocking agent potentiates the behavioural effects of 5HTP in the rat and mouse (Mantegazzini, Fabbri and Magni, unpublished observations); *c*) the depressant action of 5HTP on the electrical responses evoked at cortical and subcortical level by cutaneous or muscular afferents is enhanced by pretreatment with iproniazid (11).

Yuwiler, Geller and Eiduson (20) have shown that 5HTP inhibits, *in vitro*, the decarboxylation of DOPA to dopamine; the corresponding action *in vivo* would lead to a depletion of the catecholamine contained in the brain. DOPA produces an EEG activation in the *cerveau isolé* preparation of the cat (13) presumably by the production of catecholamine. The EEG synchronization observed with 5HTP could thus be caused by the slowing of the production of catecholamine, rather than by 5HT.

Our results differ from those of Costa and Rinaldi (6) and of Monnier and Tissot (16). The reasons for the divergence may be: *a*) interference, in the animal with intact neuraxis, between the peripheral effects and the central effects of the amino-acid; in our experiments and in some of those of Monnier and Tissot, this interference was abolished by section of the brain stem; *b*) the presence in the blood of extracerebral metabolites of 5HTP in larger amounts than in our experiments as a result of the injection of larger doses of the substance into the general circulation; *c*) the animal species employed.

In our experiments 5HTP had different EEG effects from those of 5HT. The problem is to know which of the two effects approximates more closely to the physiological one actually developed by the 5HT present in the brain. If it is admitted that the action of 5HTP is due to the 5HT which is formed in excess in the brain by decarboxylation of the amino-acid, one must conclude that it is probable that 5HTP reproduces the EEG effects of the cerebral 5HT better than the 5HT circulating in the blood.

## Summary

1) We have investigated the effects which 5-hydroxytryptamine and 5-hydroxytryptophan, injected into the carotid artery, have on the EEG record of the midpontine pretrigeminal preparation of the cat, with and without additional mesencephalic hemisection. The carotid blood-flow and the arterial pressure were recorded as a control.

2) 5HT (2-80 $\mu$g) does not alter the activated EEG record of the midpontine pretrigeminal preparation, while it desynchronizes (1-4 $\mu$g) the record in the same preparation after it has been synchronized by mesencephalic hemisection or by visual and olfactory de-afferentation. The arousal reaction does not depend on gross changes in the carotid blood-flow.

3) 5HTP (8-20 mg) synchronizes the spontaneously activated record of the midpontine pretrigeminal preparation and accentuates the synchronization of the record in the same preparation after mesencephalic hemisection. The synchronizing effect does not depend on blood-borne products of the extracerebral metabolism of the amino-acid.

4) These results support the hypothesis that the 5HT circulating in the blood has different EEG effects from those of the 5HT which is formed directly in the nervous tissue by decarboxylation of the amino-acid.


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# ACTION DE LA DL-3-4-DIOXYPHENILALANINE (DOPA) ET DE LA DOPAMINE SUR L'ACTIVITÉ ÉLECTRIQUE DU CHAT « CERVEAU ISOLÉ »

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## Introduction

Les données rapportées par Bonvallet, Dell et Hiebel (2) et par Rothballer (13) démontraient que la réaction électroencéphalographique de réveil qui suit l'injection intraveineuse d'adrénaline (ou de nor-adrénaline) se produisait par l'intermédiaire de la substance réticulée activatrice du tronc de l'encéphale, ou était en tous cas conditionnée par elle.

Longo et Silvestrini (8), Mantegazzini, Poeck et Santibanez (9) et Capon (3) observaient que l'injection d'adrénaline dans la circulation cérébrale (artère carotide ou artère vertébrale ou toutes les deux) ne reproduisait l'effet de l'injection intraveineuse que si l'on utilisait des doses proportionnellement plus élevées, et de toute façon avec une latence considérable, souvent en coïncidence avec l'hypertension systémique due au passage de l'amine dans la circulation générale. Ces deux faits étaient difficilement conciliables avec l'hypothèse émise par Dell et Bonvallet (6), selon laquelle l'amine aurait agi directement sur les neurones réticulaires; ils laissaient plutôt penser que l'activation EEG provoquée par l'adrénaline était la conséquence des effets circulatoires (systémiques ou localisés au district cérébral) ou métaboliques produits par l'amine. Ces observations concernant le mécanisme de la réaction EEG de réveil produite par l'adrénaline hématique n'apportent aucune contribution au problème de la signification fonctionnelle des catécholamines qui se trouvent dans le système nerveux central; la dopamine, la nor-

adrénaline et l'adrénaline localisées dans le cerveau pourraient intervenir dans le contrôle de l'activité électrique de l'écorce cérébrae par des mécanismes tout à fait différents de ceux vasculaires ou mlé taboliques que l'on a supposés pour les catécholamines d'origine extracérébrale. C'est à ce dernier problème qu'à été consacrée la recherche que nous rapportons ici.

L'injection de 3-4-dioxyphénylalanine (DOPA), provoque une augmentation du contenu cérébral de dopamine (5), amine considérée comme le précurseur de la nor-adrénaline et de l'adrénaline (1). Chez le lapin au névraxe intact (11) et « cerveau isolé » (10), l'injection intraveineuse de DOPA fait apparaître un tracé EEG de réveil. Nous rapportons ici des expériences effectuées chez la préparation « cerveau isolé » de chat, dans lesquelles nous avons eu pour but *a*) de comparer les effets électroencéphalographiques de la DOPA à ceux de la dopamine, produit de décarboxylation de la DOPA et *b*) d'établir si les actions vasculaires de la DOPA sont responsables de ses effets EEG.

## Méthodes

Nos expériences ont porté sur 20 chats adultes. Sous anesthésie à l'éther on exécutait la section électrolytique complète du mésencéphale selon un plan passant dorsalement par les tubercules quadrijumeaux antérieurs et ventralement par le bord antérieur du pont (préparation « cerveau isolé »). L'électrode utilisée pour la coagulation était orientée au moyen d'un appareil stéréotaxique. Entre la fin de l'administration de l'éther et le début de l'expérience on laissait s'écouler de 1.1/2 à 2 hr.

Les substances étaient injectées dans l'artère carotide externe d'un côté à travers une canule de polythène glissée à contre-courant dans l'artère linguale. Grâce à cette technique, suggérée par Desmedt et La Grutta (7), il était possible d'injecter les substances sans interrompre le flux sanguin dans l'artère utilisée pour l'injection. L'artère carotide commune du côté opposé était occluse. Pour les injections effectuées dans la circulation générale, on utilisait une veine saphène. La DOPA et la dopamine étaient dissoutes en Tyrode à la concentration de 10 mg/ml.

L'activité électrique corticale était enregistrée selon la méthode unipolaire sur un électroencéphalographe Schwarzer à 6 plumes. Les électrodes à vis étaient implantées dans l'épaisseur de la thèque, en correspondance des aires frontales, pariétales et occipitales des deux côtés. L'électrode indifférente était fixée à la peau du cou.

La pression artérielle était enregistrée à l'artère fémorale au moyen d'un électromanomètre « Elema » relié à un galvanomètre à miroir. Dans une partie des expériences on enregistrait avec la méthode de la « Thermostromuhr » de Rein les modifications du flux sanguin carotidien produites par l'injection intrartérielle de la DOPA et de la dopamine. Le thermoélément en contact avec l'artère carotide commune était relié à un second galvanomètre à miroir. Les rayons lumineux reflétés par les deux galvanomètres étaient convoyés à un photochimographe.

Les enregistrements électroencéphalographiques, du flux sanguin carotidien et de la pression artérielle étaient exécutés simultanément.

## Resultats

1. *Action de la DOPA sur le tracé EEG, sur la pression artérielle et sur le flux sanguin carotidien.* — La DOPA (8-20 mg) injectée lentement (50-200 µg/sec) dans l'artère carotide, produisait une nette désynchronisation du tracé électroencéphalographique synchronisé de la preparation « cerveau isolé » (Fig. 1 *B*). L'effet commençait 1-2 min après la fin de l'injection, durait 10-45 min et n'était jamais accompagné par quelque modification significative et stable de la pression artérielle et du flux sanguin carotidien.

L'injection intraveineuse de DOPA (2-2. 1/2 hr après l'injection intracarotidienne) à doses égales à celles qui étaient actives par voie intrartérielle, ne modifiait pas l'EEG ou tout au plus provoquait une raréfaction des fuseaux, de brève durée (Fig. 1 *C*). Cette observation démontrait que l'activation EEG provoquée par l'injection intrartérielle n'était pas due à la recirculation de l'acide aminé. Par conséquence elle ne pouvait pas être attribuée à des produits du métabolisme extracérébral de la DOPA, ni à des altérations du métabolisme extracérébral induites par l'acide aminé.

L'effet désynchronisant de l'injection intraveineuse était toutefois manifeste pour des doses supérieures d'acide aminé (30-50 mg).

2. *Action de la dopamine sur l'EEG, sur la pression artérielle et sur le flux sanguin carotidien.* — La dopamine (1-10 mg) injectée lentement (50-200 µg/sec) dans l'artère carotide ne déterminait, dans la plus grand partie des cas, aucune modification de l'activité électrique corticale synchronisée de la préparation « cerveau isolé » (Fig. 2 *A*). Exceptionnellement (2 expériences sur 15) on observait durant ou immédiatement après l'injection intracarotidienne une raréfaction des fuseaux de très bréve durée (15-40 sec).

La dopamine, aux doses indiquées et par les voies d'introduction sus-mentionnées, produisait, à la différence de la DOPA, des modifications marquées de la pression artérielle et du flux sanguin carotidien (Fig. 2 *A*). La pression artérielle augmentait de 50-120 mm Hg quelques secondes après le début de l'injection et ne rejoignait les valeurs de départ que quelques minutes après la fin de l'injection; le flux sanguin carotidien diminuait brusquement dés le début de l'injection et remontait lentement aux valeurs de départ après la fin de l'injection.

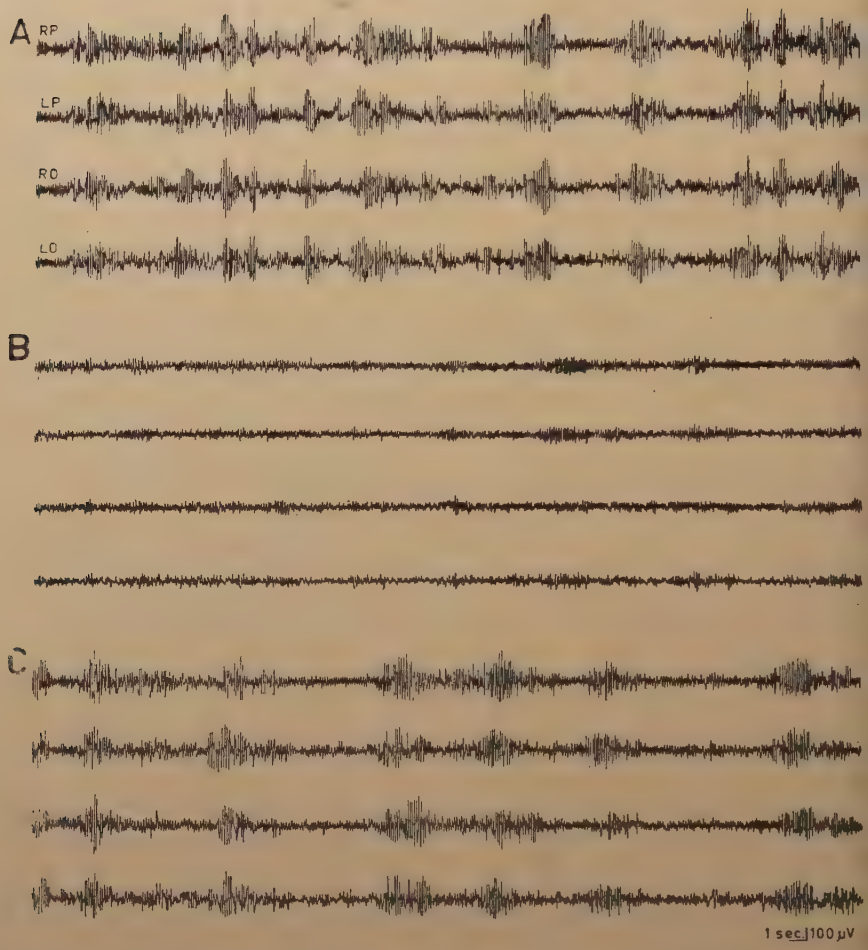


Fig. 1. – *Effets de la DOPA sur l'activité électrique corticale de la préparation « cerveau isolé » de chat.*

Dérivations, de haut en bas: pariétale droite (R. P.), pariétale gauche (L. P.), occipitale droite (R. O.), occipitale gauche (L. O.). *A*: aspect synchronisé typique de la préparation « cerveau isolé ». *B*: 5 min après la fin de l'injection de 10 mg de dl-DOPA dans l'artère carotide: aspect typique de désynchronisation corticale. *C*: 2 hr après B, et 5 min après la fin de l'injection intraveineuse de la même dose de dl-DOPA: léger espacement des fuseaux. L'effet de l'injection intraveineuse est inférieur à celui de l'injection intracarotidienne.

Dans quelques expériences de contrôle nous introduisions par voie intraveineuse de fortes quantités de dopamine (2-4 mg/min) pendant 2-3 min: la pression artérielle moyenne montait jusqu'à

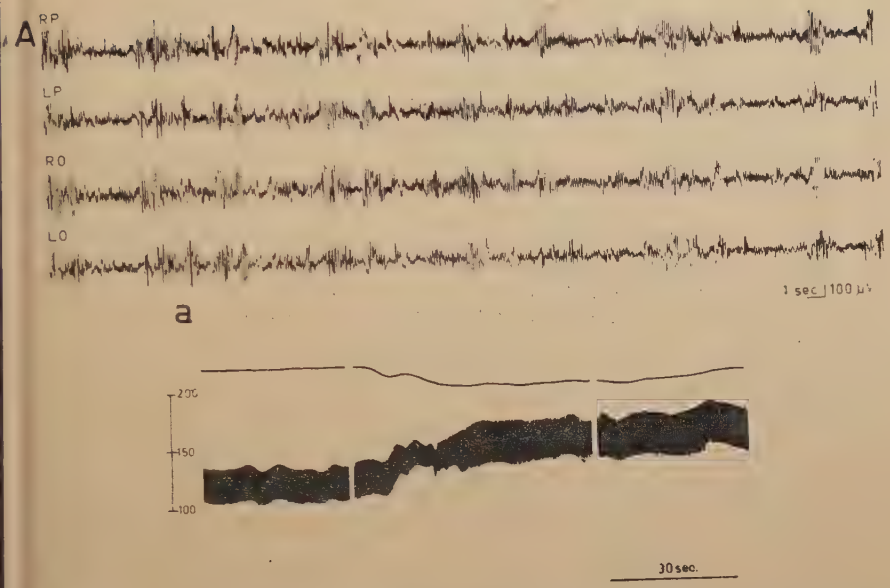


Fig. 2. – *Effets de la dopamine sur l'activité électrique corticale, sur le flux sanguin carotidien et sur la pression artérielle de la préparation « cerveau isolé » de chat.*

Suite de l'expérience rapportée dans la Fig. 1. – *A*: enregistrement de l'EEG; *a*: enregistrement du flux sanguin carotidien (en haut) et de la pression artérielle (en bas). Entre les deux signaux blancs rapportés sur les tracés du flux et de la pression, injection dans l'artère carotide de 10 mg de dopamine: le flux sanguin carotidien diminue et la pression artérielle augmente; aucune modification du tracé EEG (cf. Fig. 1 *A*). L'enregistrement EEG comence à la fin de l'injection intracarotidienne.

200-220 mm Hg, mais l'aspect électroencéphalographique restait inchangé pendant toute la durée de l'infusion.

La dopamine, à toutes les doses essayées et par les deux voies d'introduction, produisait une mydriase totale, constamment beaucoup plus marquée que celle qui suivait l'injection de DOPA.

## Discussion

La préparation utilisée, l'absence de modifications significatives de la pression artérielle et du flux sanguin carotidien après l'injection de DOPA et les différences entre les effets EEG produits par l'introduction de la même dose d'acide aminé par voie artérielle et par voie veineuse (cfr. Résultats, 1) sont des faits qui permettent

par exclusion de retenir que l'activation EEG de la DOPA est due à l'action que l'acide aminé, ou les amines auxquelles il donne lieu à l'intérieur du cerveau, exerce sur les neurones cérébraux. On peut émettre quelques doutes sur la signification des mesures du flux carotidien: en effet, le flux sanguin dans l'artère carotide commune, à pression systémique égale, dépend des résistances vasculaires au niveau des deux territoires de distribution de l'artère, l'intracérébral et l'extracérébral, et par conséquent n'est pas un signe sûr de l'action d'une substance sur la circulation cérébrale. La DOPA toutefois ne provoque aucune modification du flux: puisqu'il est improbable que les effets de l'acide aminé sur les deux territoires soient de la même grandeur mais de signe contraire, de façon à s'élider l'un l'autre parfaitement, l'on peut exclure que la DOPA dans les conditions réalisées au cours de ces expériences ait eu sur la circulation cérébrale des effets capables de modifier le tracé EEG.

La DOPA provoque dans le cerveau une augmentation du contenu de dopamine (5); le prétraitement avec iproniazide, un inhibiteur de l'aminoxydase, met en évidence également une augmentation du contenu de nor-adrénaline (4). L'une ou l'autre de ces amines, ou d'autres encore récemment mises en évidence (métanéphrine et nor-métanéphrine), ou, moins vraisemblablement, l'acide aminé lui-même, peuvent être responsables de l'effet activateur de la DOPA.

Chez la préparation « cerveau isolé », la dopamine, dans la plus grande partie des cas, ne reproduisait pas les effets EEG de la DOPA (cf. Résultats, 2). Nous comparions des doses à peu près correspondantes d'acide aminé et d'amine (8-20 mg de dl-DOPA, 1-10 mg de dopamine), de telle sorte qu'il nous est difficile de penser à un dosage insuffisant de dopamine. La différence des effets EEG des deux substances laisse croire plutôt que la dopamine d'origine extracérébrale, contrairement aux catécholamines qui se forment à partir de la DOPA à l'intérieur du cerveau, ne rejoint pas les récepteurs adrénergiques en concentration suffisante pour modifier l'activité électrique de l'écorce cérébrale.

Un certain nombre de données biochimiques démontrent que l'augmentation de la concentration hématique d'adrénaline et de nor-adrénaline n'a pas de répercussion sur le contenu cérébral d'amines correspondantes; elles permettent par conséquent d'entrevoir une indépendance fonctionelle entre les amines extracérébrales et les amines intra-cérébrales. En effet: *a*) le contenu cérébral en substances douées d'action sympathico-mimétique sur la pression ar-

térielle ne se modifie pas, chez le rat, après l'injection de fortes doses d'adrénaline et de nor-adrénaline, tandis qu'il augmente après l'administration de DOPA (12); *b*) l'extrait de cerveau de rats traités avec de l'adrénaline marquée ne manifeste pas de radioactivité (14); enfin *c*) après l'infusion intraveineuse d'adrénaline marquée chez le chat, on ne retrouve pas dans le cerveau de quantités significatives d'adrénaline et de métanéphrine marquées (16). Par contre nous n'avons pas trouvé dans la littérature de données se rapportant aux effets de l'injection de dopamine sur le contenu de catécholamines cérébrales, données qui pourraient servir de contrôle pour les conclusions que nous avons tirées de notre expérimentation, si l'on excepte l'observation de Vogt (15) selon laquelle la contenu de nor-adrénaline reste inchangé. La valeur de cette observation est diminuée toutefois par la constatation que l'introduction de DOPA n'est pas suivie par une augmentation de nor-adrénaline si l'on ne recourt auparavant à l'iproniazide (4).

## Résumé

1. Nous avons étudié les effets de la dl-3-4-dioxyphenylalanine (DOPA) et de la dopamine sur l'activité électrique corticale synchronisée de la préparation « cerveau isolé » chez le chat. Les substances étaient injectées dans une artère carotide ou dans une veine saphène. Simultanément l'on enregistrait l'EEG, la pression artérielle et le flux sanguin carotidien.

2. La DOPA (8-20 mg) injectée dans l'artère carotide produisait une nette activation corticale du tracé. A doses égales l'injection intraveineuse était moins efficace que l'injection intrartérielle. La DOPA ne produisait aucune modification significative du flux carotidien et de la pression artérielle.

3. La dopamine injectée dans l'artère carotide (1-10 mg) ou infusée dans la veine saphène (2-4 mg/min) n'apportait dans la plus grande partie des cas aucune modification du tracé EEG de la préparation « cerveau isolé », mais produisait par contre une diminution évidente du flux carotidien et une hypertension artérielle.

4. Ces résultats laissent croire: *a*) que l'activation EEG de la DOPA ne dépend ni des produits du métabolisme extracérébral de l'acide aminé, ni d'altérations de la circulation cérébrale produites

par la substance, mais qu'elle est due plutôt aux catécholamines auxquelles l'acide aminé donne lieu à l'intérieur du cerveau; *b*) que la dopamine d'origine extracérébrale ne rejoint pas les récepteurs adrénergiques sur lesquels agissent les catécholamines à localisation intracérébrale.

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# THE LIMITS OF THE $H^+$ TUBULAR TRANSPORT AND THE SITE OF THE URINE ADICIFICATION DURING METABOLIC ACIDOSIS IN DOG

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## Introduction

It is well established that the exchange mechanism between $H^+$ and $Na^+$ is related with the tubular function by which the bicarbonate-bound bases and those bound to different buffers are reabsorbed. The same process produces, however, two different consequences, viz.: 1) the reabsorption of bicarbonate-bound bases is followed by the diffusion of the corresponding $CO_2$ from the tubular urine into the tubular cells so that a minimal $H^+$ concentration gradient between plasma and urine is established; while 2) the reabsorption of the bases-bound to the different buffers determines this gradient by producing the acidification of the tubular urine, and leads to the excretion of free acid and ammonia.

When metabolic alkalosis is present there is no acid excretion, ammonia disappears from the urine and the total $H^+$ tubular transport can be represented as bicarbonate reabsorption. It is well known that in this condition the tubular capacity to transport $H^+$ is limited by a maximal rate which is identical with the maximal capacity to reabsorb bicarbonate (8).

When conversely, metabolic acidosis or a normal acid-base balance is present, so that the $H^+$ transport capacity can be partly diverted to the reabsorption of the bases bound to different buffers (7), the total $H^+$ tubular transport can be represented as the sum of bicarbonate reabsorption with the excretion of free acid and ammonia (4). The excreted ammonia appears in this sum because it

partly neutralizes the urinary acidity and thus represents the amount of the exchanged $H^+$ which does not result as free acid excretion nor as bicarbonate reabsorption.

It is known that in this condition the tubular capacity to transport $H^+$ is limited by the maximal $H^+$ concentration gradient that the tubule cells can establish between plasma and urine (11). In fact, this gradient hinders the free acid excretion so that the $H^+$ transport capacity, not required for bicarbonate reabsorption, cannot be diverted to the reabsorption of the bases bound to the other urinary buffers in an equivalent amount.

It is not known, however, whether this function is also limited by a maximal rate when, during metabolic acidosis, the $H^+$ concentration gradient is low. In other words, the question is open whether the $H^+$ tubular transport is limited by a maximal rate not only when it is involved in the reabsorption of the bicarbonate-bound bases but even when it is effecting the reabsorption of the bases bound to different buffers and the hindering action of the high H+ concentration gradient between plasma and urine is avoided.

In the present paper an attempt is described to answer this question.

As to the site of urine acidification, it is commonly believed that it is carried out in the distal convoluted tubules where an $H^+$ concentration gradient between plasma and urine can be established by the tubule cells. *In the present study, however, we have obtained data suggesting that, during metabolic acidosis, the tubular urine can be acidified, also, in the proximal tubules.*

## Methods

The present study is based on 18 experiments performed in 18 dogs, ranging in weight from 14-23 kg, which were anesthetized with sodium pentobarbital. A preliminary acidosis was induced by the oral administration of $NH_4Cl$ given in amounts ranging from 5-40 g, on the day preceeding and the morning of the day of the experiment, which was performed during the afternoon.

In 13 experiments the urinary phosphate elimination was progressively increased by giving a venous infusion of the buffer solution at increasing rates; the degree of metabolic acidosis, previously reached, was maintained as constant as possible by giving, together with the phosphate solution, an amount of HCl approximately equivalent to the renal elimination of acid for each collection period, as described in Tables 1, 2 and 3. The experimental condition was thus characterized by the glomerular filtration of bicarbonate at constant rates and by the progressively increasing elimination of phosphate. The principles underlying these experiments are

the same as those where the maximal capacity for bicarbonate reabsorption is being measured, except for the fact that phosphate is supplied, as a tubular buffer, instead of bicarbonate and that the $H^+$ tubular transport is measured as the sum of free acid and ammonia excreted and bicarbonate reabsorbed, instead of the reabsorbed bicarbonate alone.

In 5 other experiments the degree of metabolic acidosis was progressively increased by giving larger amounts of HCl, as described in Tables 4 and 5.

A 0.50 M sodium phosphate solution having a pH of 7.40 and an 0.4 N HCl solution were infused at the rates reported in the Tables. A creatinine solution (0.0 g per 100 ml) was also infused at constant rates (0.02 g per min) throughout all the experiments in order to measure the exogenous creatinine clearance.

Urine samples, at the end of each clearance period (15 minutes), and arterial plasma samples, at the half time of each two clearance periods, were collected under neutral mineral oil. Manual compression was used to avoid the incomplete emptying of the bladder. Bicarbonate, phosphate, pH and creatinine were measured in both urine and plasma samples. In the urine samples ammonia and titratable acid were also determined; this was measured by titrating back to the observed plasma pH of the corresponding collection period. The urine free acid titration, the bicarbonate determination and the pH measurements were performed within a few minutes of the time of collection; ammonia, phosphate and creatinine were determined within 24 hours and during this time the samples were kept under oil and refrigerated (0° C). Urine and plasma pH were measured anaerobically at 37° by a glass electrode (Metrohm pH-meter). Urine titration was performed by the electrometric method using 0.10 N NaOH. The plasma and urine bicarbonate concentration was calculated from the total $CO_2$ content and pH, by means of the Henderson-Hasselbalch equation using a pK′ of 6.1 for plasma and urine (8). The total $CO_2$ content was determined by the manometric method of Van Slyke and Neill (14). In the urine samples having a pH less than 6.20, bicarbonate was assumed to be absent (8). Urine and plasma phosphate concentration was measured according to the method of Fiske and Subbarow (3) slightly modified, and urinary ammonia by the aeration method of Van Slyke and Cullen (13).

The glomerular filtration rate (G. F. R.) was measured by means of the clearance of the exogenous creatinine which was determined in plasma and urine according to Bonsnes and Taussky (1). The plasma concentration values not directly determined (each two clearance periods) were calculated by interpolation.

The urine flow, which was low during the first collection periods, increased in the late stages of the experiments due to the phosphate osmotic diuresis. In order to avoid dehydration, in this stage of the experiments, an amount of fluid, approximately equal to the urine volume of the preceeding collection period, was infused during each clearance period.

## Results

All the experiments were complicated, during the late stages, by two functional alterations: the fall of the G. F. R. and the appearance of a respiratory acidosis. They were probably due to circulatory disorders and to depression of respiration, produced by phosphate intoxication. It has been demonstrated that the capacity to reabsorb bicarbonate is increased by raising the plasma $CO_2$

tension (9, 2). Since higher $pCO_2$ may also lead to an increase in the $H^+$ transport capacity in metabolic acidosis, the samples taken during the late collection periods, where these functional alterations were present, were discarded together with the samples from those periods where the fall in the G. F. R. was of great magnitude.

The other collection periods in which the G. F. R. was slightly decreased, have been included, since Thompson and Barrett (12) found that the absolute reabsorption of bicarbonate (mEq per min) remained constant even during acute reduction of G. F. R., whereas the bicarbonate reabsorption expressed in mEq per 100 ml G. F. R. rose; thus the data we obtained were expressed in mEq per min, i. e. in absolute rates. However, since this way of expressing the data does not permit their comparison with the values of the $H^+$ transport in metabolic alkalosis (bicarbonate reabsorption), which are commonly expressed in mEq per 100 ml G. F. R., the values obtained were also expressed in mEq per 100 ml of glomerular filtrate (last column of the Tables). The assumption was made in this calculation that the G. F. R. remained constant at the levels found for the first group of 3-4 clearances. This way of expressing the data does not consider the fall in the G. F. R. and it is thus independent of it.

1. *The $H^+$ transport at increasing rates of phosphate excretion, with a constant degree of metabolic acidosis.* — In Tables 1, 2 and 3 are reported three experiments with three different degrees of metabolic acidosis; from inspection of these Tables and of Fig. 1, it is evident that the $H^+$ transport during metabolic acidosis is quite similar to the other tubular functions which are limited by a maximal rate. In fact, the $H^+$ tubular transport rates, which are initially increasing, later become almost constant despite the further increase of the phosphate buffer excretion.

The reason for this behaviour of the $H^+$ tubular transport are referable to the fact that the rates of the free acid excretion, after an initial increase, become constant during the late collection periods. This is due to the fact that the tubular utilization of the buffering capacity of the excreted phosphate is progressively decreasing as is demonstrated by the increasing values of the urine pH. This is particularly evident in the experiments of Tables 2 and 3 where the degree of metabolic acidosis was lower and more constant than in the experiment of Table 1.

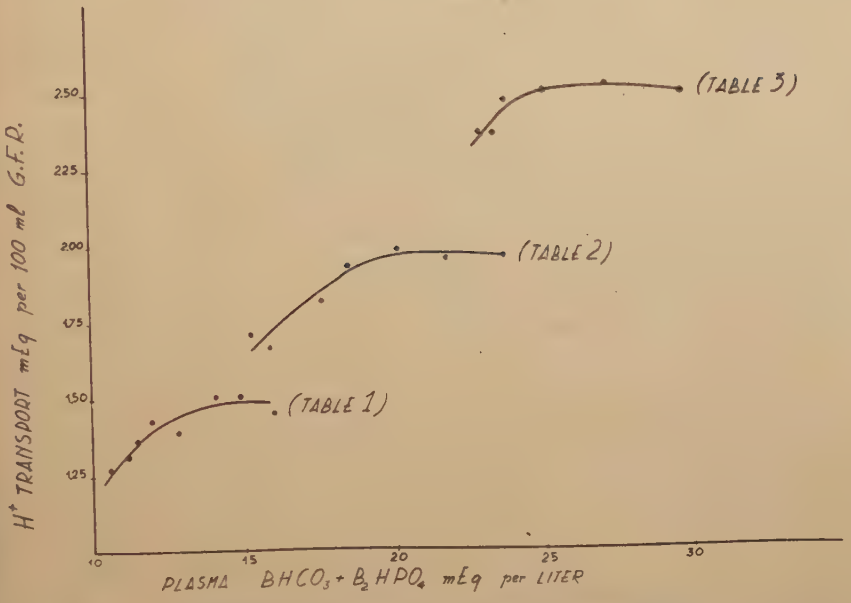


Fig. 1. – *Relationship between the plasma concentration of bicarbonate plus dibasic phosphate, and the maximal capacity to exchange H+.*

The reported values of the maximal capacity to excrete H+ are corrected to a standard value of G. F. R., by assuming that this rate remains constant throughout the experiments (the last column of the tables 1, 2 and 3). The dibasic phosphate plasma concentration was calculated from the arterial plasma pH and the plasma phosphate concentration.

2. *The relationship between $H^+$ tubular transport and arterial $pCO_2$.* — Our data show the existence of a direct correlation between the values of the plasma $pCO_2$ and the maximal rates of $H^+$ transport in metabolic acidosis (Fig. 2).

Since the values of the plasma $pCO_2$ of the reported collection periods were approximately proportional to the values of the plasma bicarbonate concentration, it follows that the less severe the degree of metabolic acidosis the higher will be the $H^+$ transport capacity. Therefore, when the degree of metabolic acidosis was very light and, consequently, the plasma $pCO_2$ values were of the same order of magnitude as those which are commonly present when the bicarbonate reabsorption is being measured, the maximal capacity to excrete $H^+$ in metabolic acidosis was also of the same order of magnitude as the maximal capacity to reabsorb bicarbonate (Fig. 1 and Table 2).

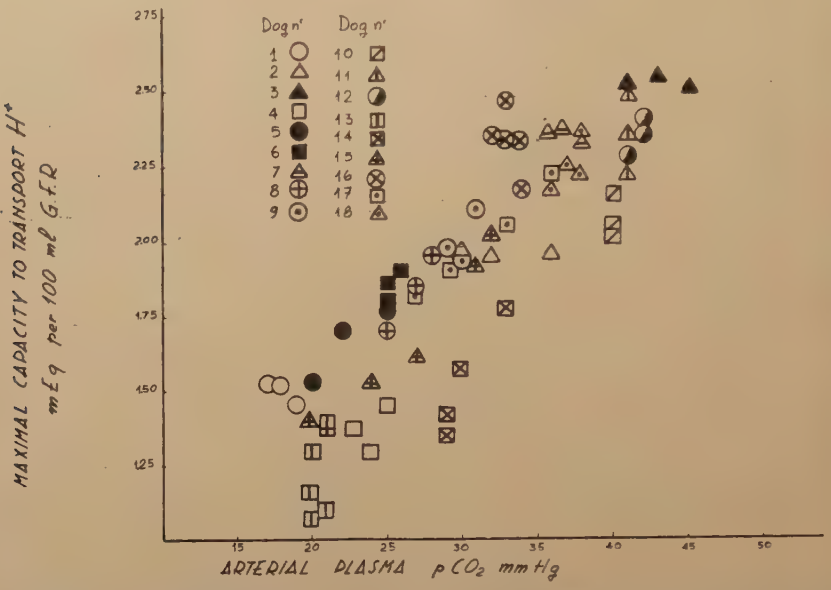


Fig. 2. – *Relationship between the arterial plasma $pCO_2$ and the maximal capacity to excrete $H+$.*

In this figure are reported the values observed in the last collection periods of the experiments corresponding to constant rates of free acid excretion.

3. *The relationship between the degree of metabolic acidosis and the free acid excretion rates.* — In Fig. 3 the maximal, fairly constant, rates of free acid excretion, observed in the late collection periods of all our experiments, are plotted against the plasma bicarbonate content of the corresponding clearance periods. From inspection of this figure it appears that, as the degree of metabolic acidosis increases, the rates of free acid excretion increase to a maximum value, which then remains constant despite further decreases of the plasma bicarbonate content and increases of the phosphate urinary excretion. It follows that, when the degree of metabolic acidosis is severe enough and the tubular load of dibasic phosphate is sufficiently large, the rates of bicarbonate reabsorption become equal to and even lower than the rates of free acid excretion (Fig. 3 and Tables 4 and 5).


Fig. 3. – *Relationship between the plasma bicarbonate concentration and the free acid excretion rates.*

The rate of bicarbonate reabsorption is obviously proportional to the plasma concentration and, therefore, it is schematized in the figure as a straight line.

## Discussion

The constancy of the values for free acid excretion in the late collection periods of the experiments described represents, in our opinion, a demonstration of the limited capacity of the tubule cells to exchange $H^+$ with the bases of the filtered phosphate buffer. It is, in fact, reasonable to think that, if the $H^+$ exchange capacity were not limited, the free acid excretion should increase together with the increasing excretion of phosphate buffer. A similar consideration leads to the conclusion that the $H^+$ transport capacity in metabolic alkalosis (i. e., the capacity to reabsorb bicarbonate) is limited, and no difference exists between the two conditions, except the nature of the buffer which is supplied.

In conclusion, we believe that the $H^+$ exchange capacity is limited not only when it is carrying out the reabsorption of the

bicarbonate-bound bases, but even when it is involved in the reabsorption of the bases bound to the phosphate buffer.

The direct correlation which we have observed between the $H^+$ transport capacity in metabolic acidosis and the plasma $pCO_2$, which is present also in metabolic alkalosis (10, 2), is in favour of such an interpretation of our results and is consistent with the statement that the available $CO_2$ is the rate-limiting factor in the $H^+$ transport capacity when plasma bicarbonate is low (10).

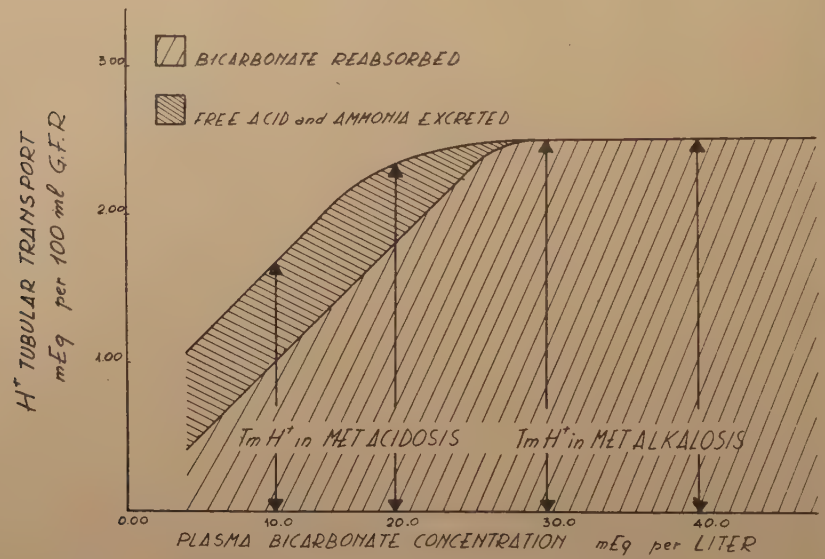


Fig. 4. – *A schematic figure where the maximal capacity to excrete H+ (TmH+) in metabolic acidosis and in metabolic alkalosis is plotted against the plasma bicarbonate concentration.*

In the schema it is assumed that the plasma $pCO_2$ in metabolic acidosis is decreasing in direct proportion to the bicarbonate concentration.

The consequences of this correlation are that, the higher the degree of metabolic acidosis (not complicated with respiratory acidosis), the lower will be the $H^+$ transport capacity (Fig. 3) and that the capacity to excrete free acid is also limited to a maximal rate without regard to the severity of metabolic acidosis and to the rates of excreted phosphate (Figs. 3 and 4).

This second consequence is not surprising if we consider that the degree of $H^+$ transport capacity, which can be diverted to the free acid excretion for the decreased load of bicarbonate (7), is ne-

cessarily constant since the $H^+$ exchange capacity ($TmH^+$) decreases proportionally to the decreasing values of the plasma bicarbonate content (Fig. 4).

These observations as a whole lead to the conclusion that the $H^+$ exchange capacity in metabolic acidosis can be considered, as in metabolic alkalosis, in terms of Tm, when phosphate is used as the bicarbonate substituting buffer.

Obviously no generalizations, concerning other buffers, are justified at present, but limiting our discussion to the phosphate, we may consider the existence, for this tubular function, of a « saturation state » and an « unsaturation state ». The former is represented by a supply of buffers (bicarbonate + bibasic phosphate) larger than the $TmH^+$ and the latter, by smaller supply.

In the « unsaturation state » (represented in the first collection periods of the experiments) the $H^+$ tubular transport is smaller than during the late stages of the experiments (Fig. 1), owing to the high $H^+$ concentration gradients which hinder the free acid excretion. Hence the entire functional potentiality of the tubule cells is not fully utilized. The full utilization occurs only in the « saturation state » and therefore in these conditions the $H^+$ transport rates are higher (Fig. 1). We can thus make the statement that in these two conditions which characterize the metabolic acidosis with respect to the renal function, the tubular excretion of $H^+$ undergoes two different rate-limiting factors: in the « saturation state », the maximal functional capacity itself ($TmH^+$) and in the « unsaturation state », the maximal $H^+$ concentration gradient that the tubule cells can establish between plasma and urine.

As to the site of urine acidification, we may venture the suggestion that the maximal observed rates of free acid excretion are so large, in respect to the reabsorbed bicarbonate, that they can not be accounted for by the distal tubule cells alone. In fact, the tubular excretion of free acid can reach values equal to and even larger than the bicarbonate reabsorption (Fig. 3). More than half the $H^+$ transport capacity can be accomplished against an $H^+$ concentration gradient and this is too large to be accounted for by the distal tubular cells alone.

In conclusion, the data obtained support the belief that, when the degree of metabolic acidosis is severe enough so that the proximal tubular cells are not reabsorbing bicarbonate, acidification of the urine takes place also in the proximal convoluted tubules.

## Summary

The tubular capacity to excrete $H^+$ in metabolic acidosis (resulting from the sum of free acid and ammonia excretion and bicarbonate reabsorption) has been studied in dogs submitted to metabolic acidosis and simultaneously loaded with increasing amounts of phosphate buffer.

The results obtained support the following conclusions.

1) In the experimental conditions employed the capacity to excrete $H^+$ is limited to a maximal rate beyond which the amount of excreted phosphate and the degree of metabolic acidosis have no influence. The maximal capacity to excrete $H^+$ ($TmH^+$) decreases with decreasing plasma $pCO_2$ values.

2) As a consequence of this correlation, when enough phosphate buffer is infused, the free acid excretion increases as the plasma bicarbonate content decreases, until a value is reached above which no further rise of free acid excretion can be obtained despite a further decrease of the plasma bicarbonate content.

3) The $H^+$ transport capacity in metabolic acidosis appears thus to be submitted to two rate-limiting factors: *a*) the maximal $H^+$ concentration gradient between plasma and urine, when the urinary buffers are low, and *b*) the maximal functional capacity ($TmH^+$), when the sum of the filtered buffers is large enough to « saturate » the $TmH^+$ itself.

4) When the degree of metabolic acidosis is severe and the phosphate excretion rate is large, the magnitude of titrable acid excretion is so large, that it can not be accounted for by the distal tubule cells alone, suggesting that urine acidification can be carried out in the proximal tubules also.


## Addendum

During the preparation of this work for publication we became aware of a paper by Gottshalk, Mylle and Laniter (5), who collected fluid from proximal convolutions of anesthetized white rats during dextrose diuresis with and without ammonium chloride loading. They observed that the proximal fluid was usually isohydric with arterial blood while fluid from the more distal convolutions was often more acid. Further acidification of the urine occurred beyond the end of the proximal convolution. Our conclusions in dog are consistent with these observations on white rats.

TABLE I.

| Time min | $PO_4$ HCl administered mM/min | | G.F.R. ml/min | Urine flow ml/min | Plasma | | | | Urine | | | | Bic. reabs. | Free acid. tub. excr. | $NH_3$ excr. | $H^+$ transp. | $H^+$ transp. mEq per 100 ml G.F.R. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | pH | $BHCO_3$ mEq/l. | $pCO_2$ mm/Hg | $PO_4$ mM/l. | pH | Titr. ac. mEq/l. | $NH_3$ mEq/l. | $PO_4$ mM/l. | mEq/min | | | | |
| 15 | — | 0.26 | — | — | 7.35 | 10.0 | 18 | 0.64 | — | — | — | — | — | — | — | — | — |
| 30 | — | 0.26 | 68 | 1.40 | 7.34 | 10.0 | 17 | 0.69 | 5.32 | 55 | 41 | — | 0.71 | 0.08 | 0.06 | 0.85 | 1.27 |
| 45 | 0.16 | 0.26 | 68 | 1.52 | 7.33 | 10.0 | 18 | 1.55 | 5.25 | 72 | 42 | 29 | 0.71 | 0.11 | 0.06 | 0.88 | 1.31 |
| 60 | 0.33 | 0.26 | 66 | 2.00 | 7.32 | 9.5 | 17 | 2.54 | 5.10 | 98 | 28 | 35 | 0.66 | 0.19 | 0.06 | 0.91 | 1.36 |
| 75 | 0.50 | 0.40 | 68 | 3.66 | 7.31 | 9.0 | 16 | 3.80 | 5.30 | 66 | 22 | 42 | 0.64 | 0.24 | 0.08 | 0.96 | 1.43 |
| 90 | 0.66 | 0.40 | 66 | 5.86 | 7.29 | 8.7 | 16 | 5.48 | 5.62 | 44 | 12 | 40 | 0.60 | 0.26 | 0.07 | 0.93 | 1.39 |
| 105 | 0.83 | 0.40 | 65 | 7.66 | 7.27 | 8.4 | 17 | 7.52 | 5.80 | 49 | 8 | 48 | 0.58 | 0.37 | 0.06 | 1.01 | 1.51 |
| 120 | 1.00 | 0.80 | 64 | 9.06 | 7.24 | 8.2 | 18 | 9.03 | 5.98 | 45 | 6 | 48 | 0.55 | 0.41 | 0.05 | 1.01 | 1.51 |
| 135 | 1.16 | 0.80 | 60 | 8.00 | 7.20 | 8.0 | 19 | 10.96 | 5.55 | 53 | 6 | 67 | 0.50 | 0.42 | 0.05 | 0.97 | 1.45 |

The H+ transport per 100 ml of glomerular filtrate (last column of the table) was calculated by assuming that G. F. R. remained constant at a value of 67 ml/min.

Bicarbonate was absent from the urine samples and the reabsorbed bicarbonate was, therefore, equal to the filtered amount; this was calculated as the product of the G. F. R. and bicarbonate plasma concentration which was corrected for the Donnan factor of 1.05.

TABLE 2.

| Time min | $PO_4$ HCl administered mM/min | | G.F.R. ml/min | Urine flow ml/min | Plasma | | | | Urine | | | | Bic. reabs. | Free acid. tub. excr. | $NH_3$ excr. | $H^+$ transp. | $H^+$ transp. mEq per 100 ml G.F.R. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | pH | $BHCO_3$ mEq/l. | $pCO_2$ mm/Hg | $PO_4$ mM/l. | pH | Titr. ac. mEq/l. | $NH_3$ mEq/l. | $PO_4$ mM/l. | mEq/min | | | | |
| 15 | — | — | 72 | 0.80 | 7.31 | 12.4 | 24 | 0.48 | 6.50 | 45 | 100 | 6 | 0.93 | 0.04 | 0.08 | 1.05 | 1.46 |
| 30 | 0.16 | 0.26 | 74 | 1.06 | 7.31 | 12.6 | 25 | 0.52 | 6.05 | 58 | 80 | 21 | 0.98 | 0.06 | 0.08 | 1.12 | 1.55 |
| 45 | 0.33 | 0.26 | 71 | 0.93 | 7.31 | 13.0 | 26 | 1.50 | 5.76 | 60 | 110 | 32 | 0.97 | 0.06 | 0.10 | 1.13 | 1.57 |
| 60 | 0.50 | 0.26 | 70 | 1.26 | 7.31 | 13.4 | 26 | 2.50 | 5.47 | 85 | 100 | 56 | 0.99 | 0.11 | 0.13 | 1.23 | 1.71 |
| 75 | 0.66 | 0.26 | 72 | 1.06 | 7.30 | 13.2 | 27 | 3.52 | 5.40 | 132 | 60 | 101 | 1.00 | 0.14 | 0.06 | 1.20 | 1.67 |
| 90 | 0.83 | 0.40 | 69 | 2.93 | 7.30 | 13.9 | 28 | 4.80 | 5.70 | 81 | 20 | 85 | 1.01 | 0.24 | 0.06 | 1.31 | 1.82 |
| 105 | 1.00 | 0.40 | 69 | 6.66 | 7.30 | 13.7 | 28 | 6.20 | 6.02 | 55 | 6 | 56 | 0.99 | 0.37 | 0.04 | 1.40 | 1.94 |
| 120 | 1.16 | 0.40 | 67 | 10.00 | 7.29 | 14.0 | 30 | 8.00 | 6.15 | 40 | 4 | 46 | 0.99 | 0.40 | 0.04 | 1.43 | 1.99 |
| 135 | 1.16 | 0.40 | 64 | 10.46 | 7.28 | 14.4 | 32 | 9.68 | 6.25 | 38 | 4 | 54 | 0.97 | 0.40 | 0.04 | 1.41 | 1.96 |
| 150 | 1.16 | 0.40 | 61 | 10.00 | 7.24 | 14.9 | 36 | 11.80 | 6.25 | 43 | 4 | 70 | 0.95 | 0.43 | 0.04 | 1.42 | 1.97 |

The $H^+$ transport per 100 ml of glomerular filtrate (last column of the table) was calculated by assuming the G. F. R. to remain constant at a value of 72 ml/min.

For other indications see table 1.

TABLE 3.

| TIME min | $PO_4$ HCl administered mM/min | | G.F.R. ml/min | URINE FLOW ml/min | PLASMA pH | PLASMA $BHCO_3$ mEq/l. | PLASMA $pCO_2$ mm/Hg | PLASMA $PO_4$ mM/l. | URINE pH | URINE TITR. AC. mEq/l. | URINE $NH_3$ mEq/l. | URINE $PO_4$ mM/l. | BIC. REABS. mEq/min | FREE ACID. TUB. EXCR. mEq/min | $NH_3$ EXCR. mEq/min | H+ TRANSP. mEq/min | H+ transp. mEq per 100 ml G.F.R. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | — | — | — | — | 7.38 | 22.2 | 41 | 0.60 | 5.68 | — | — | — | — | — | — | — | — |
| 30 | 0.16 | — | 62 | 0.32 | 7.38 | 22.3 | 41 | 0.90 | 5.86 | 75 | 150 | 51 | 1.45 | 0.02 | 0.05 | 1.52 | 2.38 |
| 45 | 0.33 | — | 61 | 1.06 | 7.37 | 22.2 | 42 | 1.50 | 6.20 | 46 | 44 | 45 | 1.42 | 0.05 | 0.05 | 1.52 | 2.38 |
| 60 | 0.50 | 0.12 | 63 | 1.56 | 7.37 | 22.1 | 42 | 2.10 | 6.59 | 36 | 30 | 43 | 1.46 | 0.06 | 0.05 | 1.57 | 2.49 |
| 75 | 0.66 | 0.26 | 63 | 3.73 | 7.37 | 22.0 | 41 | 3.80 | 6.71 | 24 | 10 | 45 | 1.46 | 0.09 | 0.04 | 1.59 | 2.52 |
| 90 | 0.83 | 0.40 | 61 | 7.33 | 7.35 | 22.3 | 43 | 6.10 | 6.74 | 19 | 4 | 40 | 1.43 | 0.14 | 0.03 | 1.60 | 2.54 |
| 105 | 1.00 | 0.40 | 58 | 8.20 | 7.33 | 22.6 | 45 | 0.05 | 6.78 | 22 | 4 | 60 | 1.37 | 0.18 | 0.03 | 1.58 | 2.51 |

The H+ transport per 100 ml of glomerular filtrate (last column of the table) was calculated assuming the G. F. R. to remain constant at a value of 63 ml/min.

For other indications see table 1.

TABLE 4.

| Time min | $PO_4$ HCl administered mM/min | | G.F.R. ml/min | Urine flow ml/min | Plasma | | | | Urine | | | | Bic. reabs. | Free acid. tub. excr. | $NH_3$ excr. | $H^+$ transp. | $H^+$ transp. mEq per 100 ml G.F.R. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | pH | $BHCO_3$ mEq/l. | $pCO_2$ mm/Hg | $PO_4$ mM/l. | pH | Titr. ac. mEq/l. | $NH_3$ mEq/l. | $PO_4$ mM/l. | mEq/min | | | | |
| 15 | — | 0.66 | — | — | 7.28 | 14.5 | 32 | 1.50 | — | — | — | — | — | — | — | — | — |
| 30 | 0.16 | 0.66 | — | — | 7.24 | 13.5 | 31 | 1.50 | — | — | — | — | — | — | — | — | — |
| 45 | 0.33 | 0.66 | 41 | 0.66 | 7.20 | 12.5 | 32 | 2.00 | 5.96 | 17 | 272 | — | 0.53 | 0.01 | 0.18 | 0.72 | 1.71 |
| 60 | 0.33 | 0.66 | 41 | 0.60 | 7.17 | 11.5 | 32 | 2.50 | 5.90 | 25 | 300 | — | 0.49 | 0.02 | 0.18 | 0.69 | 1.69 |
| 75 | 0.50 | 0.66 | 43 | 1.20 | 7.12 | 10.0 | 30 | 3.70 | 5.71 | 68 | 150 | 11 | 0.45 | 0.08 | 0.18 | 0.71 | 1.69 |
| 90 | 0.50 | 0.66 | 42 | 1.40 | 7.11 | 9.0 | 27 | 4.90 | 5.71 | 78 | 71 | 17 | 0.40 | 0.11 | 0.10 | 0.61 | 1.45 |
| 105 | 0.50 | 0.66 | 43 | 1.53 | 7.10 | 8.0 | 25 | 5.60 | 5.73 | 79 | 65 | 20 | 0.36 | 0.12 | 0.10 | 0.58 | 1.38 |
| 120 | 0.66 | 0.66 | 38 | 2.00 | 7.08 | 7.0 | 22 | 7.50 | 5.75 | 68 | 40 | 27 | 0.28 | 0.14 | 0.08 | 0.50 | 1.19 |
| 135 | 0.66 | 0.93 | 46 | 2.66 | 7.06 | 6.5 | 22 | 9.10 | 5.83 | 60 | 30 | 33 | 0.32 | 0.16 | 0.08 | 0.56 | 1.33 |
| 150 | 0.66 | 0.93 | 43 | 4.86 | 7.03 | 6.0 | 21 | 11.00 | 5.94 | 50 | 14 | 64 | 0.27 | 0.24 | 0.07 | 0.58 | 1.38 |
| 165 | 0.83 | 0.93 | 42 | 8.33 | 7.00 | 5.7 | 21 | 13.90 | 5.99 | 32 | 8 | 47 | 0.25 | 0.27 | 0.07 | 0.59 | 1.40 |
| 180 | 0.83 | 1.20 | 43 | 8.46 | 6.98 | 5.0 | 20 | 16.00 | 6.00 | 30 | 9 | 44 | 0.22 | 0.25 | 0.08 | 0.55 | 1.31 |
| 195 | 0.83 | 1.20 | 29 | 7.20 | 6.96 | 4.8 | 20 | 17.10 | 5.99 | 38 | 10 | 49 | 0.15 | 0.27 | 0.07 | 0.49 | 1.17 |
| 210 | 1.00 | 1.20 | 31 | 7.60 | 6.90 | 4.5 | 20 | 22.00 | 5.99 | 31 | 9 | 55 | 0.15 | 0.24 | 0.07 | 0.46 | 1.09 |
| 225 | 1.00 | 1.46 | 30 | 8.53 | 6.87 | 4.3 | 21 | 23.50 | 6.00 | 29 | 9 | 64 | 0.14 | 0.25 | 0.08 | 0.47 | 1.12 |

The H+ transport per 100 ml of glomerular filtrate (last column of the table) was calculated assuming the G. F. R to remain constant at a value of 42 ml/min.

For other indications see table 1.

TABLE 5.

| Time min | $PO_4$ HCl administered mM/min | | G.F.R. ml/min | Urine flow ml/min | Plasma | | | | Urine | | | | Bic. reabs. | Free acid. tub. excr. | $NH_3$ excr. | $H^+$ transp. | $H^+$ transp. mEq per 100 ml G.F.R. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | pH | $BHCO_3$ mEq/l. | $pCO_2$ mm/Hg | $PO_4$ mM/l. | pH | Titr. ac. mEq/l. | $NH_3$ mEq/l. | $PO_4$ mM/l. | mEq/min | | | | |
| | 0.80 | 0.50 | 94 | 0.93 | 7.37 | 20.0 | 37 | 2.58 | 6.30 | 80 | 42 | 129 | 1.88 | 0.07 | 0.04 | 1.99 | 2.08 |
| | 0.80 | 0.50 | 96 | 1.00 | 7.36 | 19.1 | 36 | 3.50 | 6.20 | 87 | 42 | 185 | 1.83 | 0.09 | 0.04 | 1.96 | 2.05 |
| | 0.80 | 0.50 | 96 | 1.16 | 7.35 | 18.7 | 36 | 4.19 | 6.10 | 87 | 52 | 185 | 1.89 | 0.13 | 0.05 | 2.07 | 2.17 |
| | 0.80 | 0.50 | 95 | 1.33 | 7.34 | 18.2 | 36 | 5.15 | 6.06 | 147 | 42 | 175 | 1.72 | 0.19 | 0.05 | 1.96 | 2.05 |
| | 1.06 | 0.66 | 94 | 1.80 | 7.33 | 17.6 | 35 | 7.41 | 6.05 | 145 | 20 | 153 | 1.65 | 0.26 | 0.04 | 1.95 | 2.04 |
| | 1.06 | 0.66 | 96 | 1.73 | 7.31 | 17.2 | 36 | 8.75 | 6.06 | 185 | 15 | 129 | 1.65 | 0.32 | 0.03 | 2.00 | 2.10 |
| | 1.06 | 0.66 | 93 | 3.80 | 7.30 | 16.1 | 34 | 10.32 | 6.02 | 110 | 8 | 120 | 1.59 | 0.41 | 0.03 | 2.03 | 2.13 |
| | 1.33 | 1.00 | 94 | 4.53 | 7.29 | 15.0 | 32 | 12.45 | 6.05 | 90 | 7 | 97 | 1.41 | 0.40 | 0.03 | 1.84 | 1.93 |
| | 1.33 | 1.00 | 92 | 6.06 | 7.28 | 14.6 | 32 | 13.22 | 6.05 | 74 | 5 | 97 | 1.34 | 0.45 | 0.03 | 1.82 | 1.91 |
| | 1.33 | 1.00 | 89 | 7.33 | 7.25 | 14.2 | 34 | 16.50 | 6.09 | 71 | 5 | 104 | 1.26 | 0.52 | 0.04 | 1.82 | 1.91 |
| | 1.60 | 1.50 | 90 | 7.00 | 7.23 | 13.4 | 33 | 18.70 | 6.15 | 70 | 5 | 100 | 1.20 | 0.49 | 0.04 | 1.73 | 1.81 |
| | 1.60 | 1.50 | 88 | 9.46 | 7.19 | 12.4 | 33 | 19.16 | 6.19 | 61 | 3 | 104 | 1.19 | 0.57 | 0.03 | 1.79 | 1.87 |
| | 1.60 | 1.50 | 85 | 10.73 | 7.17 | 11.8 | 32 | 21.93 | 6.17 | 60 | 3 | 104 | 1.00 | 0.64 | 0.03 | 1.67 | 1.75 |
| | 1.86 | 2.00 | 88 | 11.33 | 7.13 | 11.2 | 33 | 24.00 | 6.14 | 60 | 3 | 112 | 0.98 | 0.68 | 0.03 | 1.69 | 1.77 |
| | 1.86 | 2.00 | 85 | 12.66 | 7.08 | 9.1 | 30 | 27.50 | 6.16 | 56 | 3 | 109 | 0.77 | 0.71 | 0.03 | 1.51 | 1.58 |
| | 1.86 | 2.00 | 82 | 10.80 | 7.04 | 8.0 | 29 | 32.60 | 6.18 | 62 | 3 | 88 | 0.65 | 0.67 | 0.03 | 1.35 | 1.41 |
| | 2.13 | 2.50 | 78 | 11.73 | 6.98 | 7.2 | 29 | 34.17 | 6.16 | 60 | 2 | 116 | 0.57 | 0.70 | 0.02 | 1.29 | 1.35 |

The $H^+$ transport per 100 ml of glomerular filtrate (last column of the table) was calculated assuming the G. F. R. to remain constant at a value of 95 ml/min.

For other indications see table 1.

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# THE EFFECT OF SOME SYNAPTIC POISONS AND TRANSMITTERS ON THE INCREASE OF THE ELECTRORETINOGRAM OBTAINED BY INJECTIONS OF BARBITURATES

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## Introduction

Recent work (5, 6, 14, 19, 20) has demonstrated that some substances, among them nembutal (pentobarbitone), characteristically influence the electroretinogram (ERG). With sufficiently strong test lights as stimuli both a- and b-wave undergo a remarkable increase while with weak stimuli a decrease of the b-wave has been observed (6, 20), there being no a-wave present at low intensities in the mammals used (cat, rabbit, rat). Both effects, independently discovered by Wohlzogen and Danis in the same year (1956), have been confirmed in the present work which is devoted mainly to strong lights and the increase of the initial waves of the ERG. Since the increase comprises both a- and b-wave, it is not likely to be due to a selective effect on either component with the other merely coming to the fore as a consequence of algebraical interference between two opposite changes of potential. The reduction of the b-wave of the ERG has also been seen by Noell (19). Apparently the effects are complex since in flicker with strong lights the b-wave may decrease at a time when the response to single flashes is increased (1).

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The theoretical explanations offered by both Wohlzogen (20) and Danis (6) for the effects of nembutal on the single-flash ERG follow the same pattern. Danis imagines that between receptors and bipolars there might be a system exercising a permanent or tonic inhibition on the ERG which therefore would be " released " by nembutal. Wohlzogen requires from the same system two different effects depending upon stimulus intensity, while Danis ascribes variations with stimulus intensity to varying temporal onset of the component responses initiating the a- and b-wave. Jacobson and Gestring (14) have postulated another type of neural control, emanating from centrifugal fibres, but since the effect of nembutal is seen in decerebrate animals (1, 6) their hypothesis can hardly be regarded as necessary.

A brief report will be given below of some experiments in which the idea of a neural control of the size of the ERG is subjected to a test with the aid of some well-known synaptic poisons and transmitters. Myanesin ($\alpha$, $\beta$-dihydroxy-$\gamma$-(2 methylphenoxy)-propane) was chosen because of its depressant action on polysynaptic reflexes (2, 3, 13, 15). In view of the evidence from histochemical work to the effect that true cholinesterase is found in the nervous centre of the retina (10, 12, 16, 17, 18), supported also by chemical work (8, 9), it was felt worth while also to try eserine, acetylcholine and dihydro-$\beta$-erythroidine-hydrobromide (DHE). Some difference of opinion exists in the matter of where the retinal cholinesterase is localized, some authors (10) holding it to be found only in the synapses between bipolar and ganglion cells, others (12, 16) finding it also in the cells themselves (bipolars, ganglion cells). Eserine and DHE have powerful effects on the Renshaw cells on to which the recurrent collaterals of Golgi run from the axons of ventral horn cells (7). Eserine excites these cells, DHE suppresses their activity.

The programme of the present work, in accordance with what has been stated, was therefore to find out if the effect of nembutal on the early phase of the ERG could be significantly modified by myanesin, eserine, acetylcholine or DHE. Each experiment began with establishing the effect on the ERG of an intra-arterial injection of a small dose of nembutal. This test was then repeated after injections of anyone of the other substances mentioned, controls being done with saline.

Barbiturate has always been administered through the carotid artery as was done by Danis and Wohlzogen (6, 20). With this kind

of administration it is possible to use smaller quantities of the drug and, at the same time, to obtain a stronger effect on the retina and smaller general reactions. The other substances were generally injected intravenously, in some experiments by the intra-arterial route.

## Methods

Pigmented adult rabbits were employed throughout this investigation. In some experiments an "encéphale isolé" preparation was used but generally the animals were anaesthetized by an injection of a mixture of 1% chloralose and 10% urethane (5 ml/kg; 1/3 intravenously and 2/3 intraperitoneally). Under light ether anaesthesia a polyethylene cannula was introduced into the femoral vein while a second cannula was connected to the femoral artery; the blood pressure was continuously recorded by an Elema manometric bridge.

For the injections into the carotid a cannula was inserted into the left maxillaris externa artery in such a way that the injected drug reached the blood stream of the left external carotid. The distal end of this one and the left lingual artery were tied off.

The animal was put into a stereotactic head holder; the nictitating membrane was removed and the eyelids were held apart by stay sutures.

To registrate the ERG, mydriasis was achieved by the instillation of a drop of 1% atropine and 2% isodrine. The electrodes were calomel half cells and they were connected to left cornea and nose. The animals were dark-adapted for at least 1 hour. Shortly before the experiments were begun, Flaxedil was given (4 mg/kg/90 min intravenously) and artificial respiration introduced. The electrical equipment we have used has been described by several workers in this laboratory (see Arden, Granit and Ponte, 1). The light source was a 6 V, 15 A tungsten ribbon lamp run at a constant colour temperature of 2760° *K*. The beam passed through a shutter and through steel powder neutral filters (Bausch and Lomb) for reduction of intensity. The filament was finally focused on the rabbit's cornea so that a bright image of the last lens was seen in Maxwellian view subtending 14° of visual angle. However, the effective stimulus is spread all over the retina (e. g., Boynton, 4). The calibration of the light is described in a previous paper (1). As a rule the flash duration was 100 msec but in some experiments longer and shorter flashes were also tested.

## Results

1. *Effects of Nembutal on the ERG.* — The intra-arterial injection of nembutal (pentobarbitone) had different effects on the rabbit's ERG depending upon the light intensity that was used. Figs. 1 and 2 show the phenomenon with high and low intensities of light stimulation respectively; in both experiments the flash duration was 100 msec and 5 mg nembutal/kg were injected.

With high intensity (Fig. 1) a transient increase of amplitude and latency of both a- and b-wave was observed, proportionally greater for the a-wave (increase 38%) than for the b-wave (27%).

These variations were immediate, lasted for a duration of 15-20 sec, so that latency and amplitude of the a- and b-wave again became definitely normal 2-3 min after the injection. The opposite effect was seen with low intensity of the light stimulus (Fig. 2); the b-wave was strongly depressed or abolished (there is no a-wave with low intensity) and after 15-20 sec, recovery began and was completed in

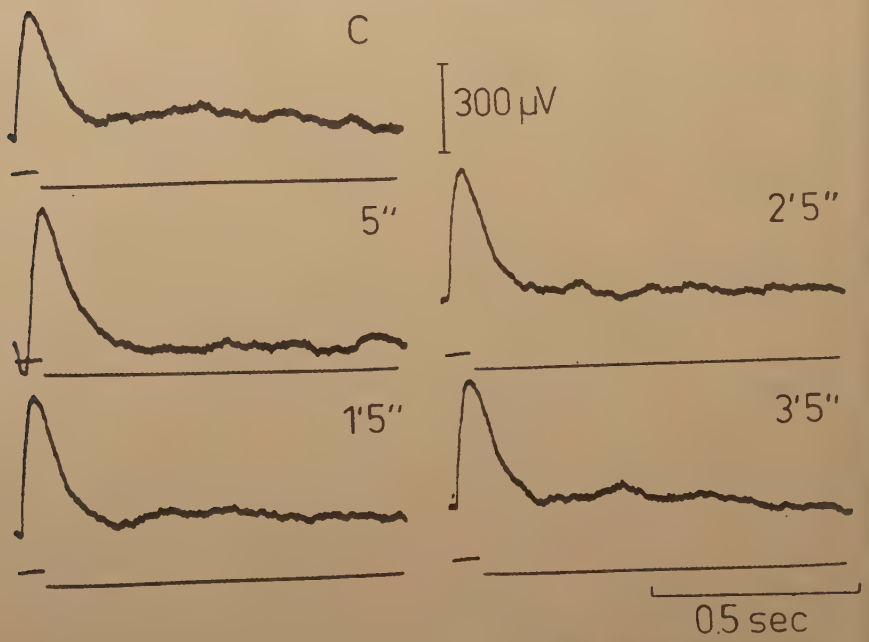


Fig. 1. – *Effect of nembutal on rabbit's ERG to low intensity of light stimulation.*

Control record was taken immediately before the injection of 5 mg/kg i. a.; the others at the times marked. Photocell monitor, lower trace, up = on. Stimulus intensity 80,000 lux.

3-4 min. The intensity of the light stimulus necessary to obtain inversion of the nembutal effect from increase to decrease of the ERG, was different from animal to animal. In this study the decrease of the b-wave was always seen with intensities below 30,000 lux, an increase of both a- and b-wave from intensities above 75,000 lux; variable responses were observed with intensities in between.

Only in two animals was the electroretinographic response to nembutal a decrease of the b-wave in spite of the very high light

intensity tried. We have no explanation of the exceptional behaviour of these two preparations.

The nembutal effect with any one light intensity was well reproducible in one and the same animal. However, when repeating injections several times, it was commonly observed that, with high stimulus intensity, the variations of the ERG tended to become irregular, both with respect to a- and b-wave. Moreover, a depression of the b-wave often succeeded the usual increase; the recovery was delayed up to 8-10 min. This is illustrated in Fig. 3 referring to a rabbit in which four injections of 5 mg nembutal/kg every 20 min

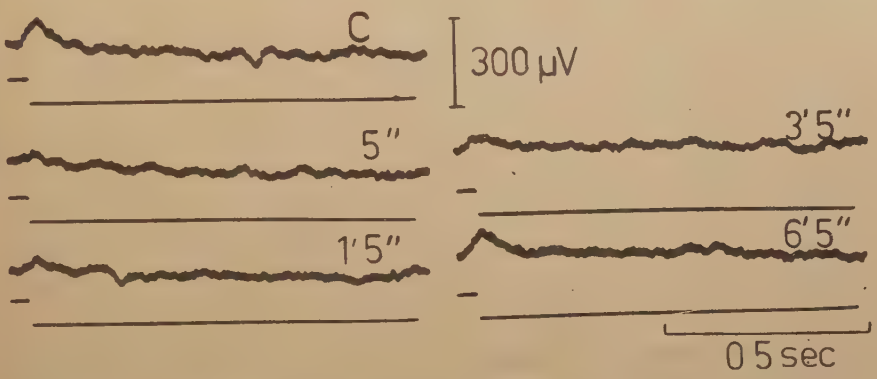


Fig. 2. – *Effect of nembutal on rabbit's ERG with low intensity of light stimulation.*

Marked as in Fig. 1. Stimulus intensity 240 lux.

preceded the one illustrated; light intensity was 75,000 lux. Five sec after the fifth injection of nembutal (5 mg/kg) the usual increase of both a- and b-wave was observed but immediately afterwards depression set in and lasted for several minutes. The ERG became normal after 11 min.

In this animal the blood pressure fell from 90 to 75 mm/Hg immediately after the injection and returned to the initial value after about 10 min. A drop of blood pressure such as the one seen in this experiment, accompanied depressions of the kind described and may therefore be responsible for the results. In fact, it is well known that the barbiturates in doses of the order used here, have but insignificant effects on the cardiovascular system but repetition will bring the total quantity injected to a level at which depression

of the vasomotor centres has been observed (11). In this work it has been found that repeated intra-arterial injections of 5 mg/kg after the fourth or fifth time cause irregularities in both ERG and blood pressure response.

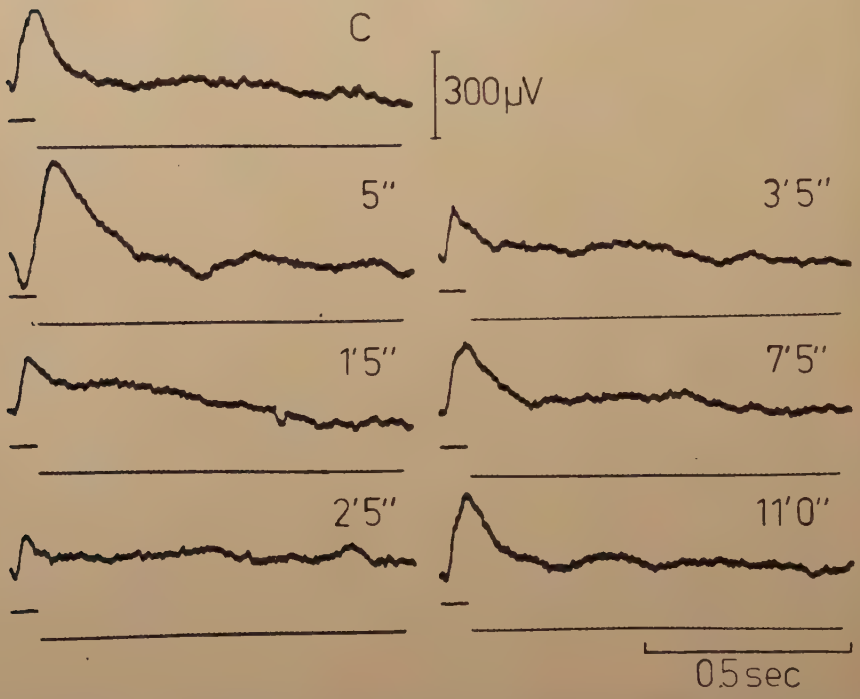


Fig. 3. – *Depression following the first, immediate increase of latency and amplitude of a- and b-wave with repeated injections of nembutal, 5 mg/kg i.a.*

In this experiment 4 previous injections of the barbiturate at intervals of 20 min preceded the illustrated one. Stimulus intensity 75,000 lux. Blood pressure decreased from 90 to 75 mm Hg after the injection. Marked as in Fig. 1.

2. *Effects of dihydro-β-erythroidine* (*DHE*). — Neither by the intravenous nor by the intra-arterial route has this drug had any definite action on the ERG. Intra-arterial injection of doses from 0.05 to 0.1 mg DHE/kg sometimes produced a fast and small increase of the b-wave (Fig. 4) but since the same effect was seen also after the controls with saline they cannot be regarded as specific. They probably follow from local modifications of blood flow. In Danis' extensive work (6) small variations of the amplitude of the b-wave

after similar injections of saline were also reported. The intravenous injections comprised doses from 0.1 to 0.5 mg/kg.

In the graph of Fig. 4 amplitudes of the a- and b-wave have been plotted against running time as abscissa. In this experiment DHE, nembutal and saline were given to a rabbit in chloralose-

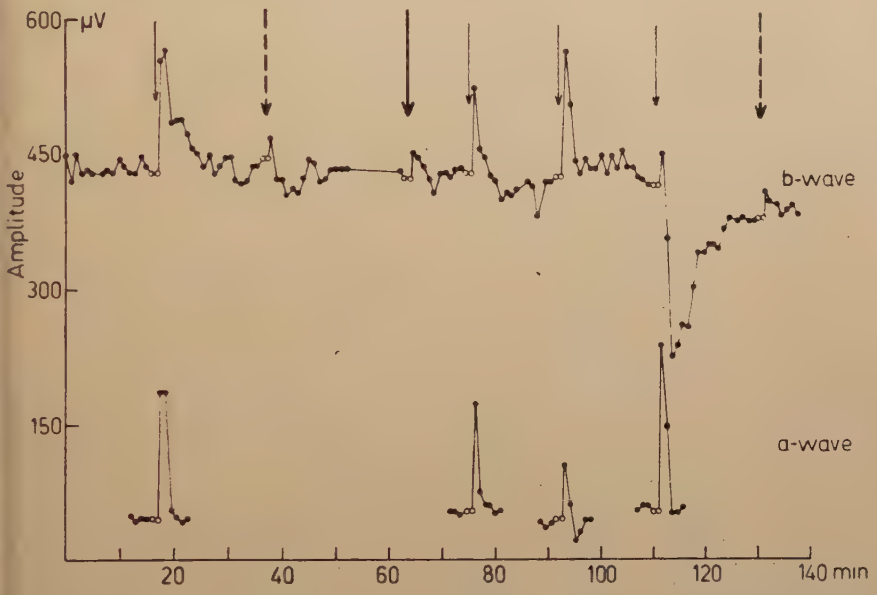


Fig. 4. – *Usual effect of nembutal on rabbit's ERG after injection of dihydro-β-erythroidine.*

Chloralose-urethane rabbit, 3 kg. Amplitude of a- and b-wave plotted against time from the beginning of the experiment. Stimulus intensity 75.000 lux. Slender arrows, injections of nembutal, 10 mg/kg i. a.; thick arrow, injection of DHE, 0.1 mg/kg i. v.; dashed arrows, injections of isotonic saline solution, 0.3 ml i. a., corresponding to the quantity of the injected fluid containing nembutal. Open circles mark duration of the injections (1 min); the first filled circle is always at 30 sec after each injection; the others, as a rule, follow once every minute. No variation of the a-wave after the injections of DHE and saline; therefore only relevant variations preceded by some controls have been illustrated.

urethane anaesthesia. The intensity of light stimulation was 75,000 lux. The first injection of nembutal (5 mg/kg) was followed by the usual increase of both a- and b-wave. After the injection of DHE or saline there was a small transient increase of the b-wave alone. The effect of nembutal was not significantly modified after DHE. The b-wave after the last injection of nembutal was depressed

(cf. above, for effect of repeated injections of nembutal at short intervals). After some time the normal effect of nembutal on the ERG returned.

3. *Effects of Myanesin on the ERG.* — Some experiments were devoted to a study of the effects of myanesin on the ERG. The injected doses were 10 and 30 mg/kg i. a. or 30 and 60 mg/kg i. v. A fall of the blood pressure from 90 to 60 mm Hg in the first 4-5 min from the intravenous injection was always present, even when the precaution was taken to inject very slowly at constant speed over one minute. In five experiments the drug was given intravenously. In four of them, independently of the intensity of the light used, there was a moderate decrease of the b-wave which recovered in 4-5 min, while a slight increase occurred in one experiment only. It seems therefore that myanesin has no clear specific effect on the ERG. In fact, the common slight reduction of the ERG is best explained by the fall of blood pressure. In two other animals the drug was injected intra-arterially. Only a transitory non-specific increase of the b-wave was observed similar to the effect of an intra-arterial injection of the same quantity of saline.

Fig. 5 shows the results of one experiment in which intra-arterial injections of nembutal were made from the 5th to the 50th min following an intravenous injection of 30 mg/kg myanesin. Three successive tests with nembutal were carried out 45 min before myanesin was given. The modest effect of the first injection of nembutal five min after myanesin, may be a consequence of the drop of blood pressure. The depressant action of myanesin on the polysynaptic pathways in the central nervous system generally lasts from 20 to 180 min (15) while in this experiment the characteristic effect of nembutal returned within 35 min. The blood pressure, however, was normalized within 10 min and so a small genuine depression of the ERG cannot be wholly excluded.

4. *Effects of eserine sulphate and acetylcholine on the ERG.* — Both eserine and acetylcholine were ineffective on the ERG of the rabbit. Eserine was injected intravenously in doses of 0.5 mg/kg and intra-arterially in doses of 0.1 mg/kg while acetylcholine was given intra-arterially in doses of 0.1 mg/kg. In every experiment recording of the ERG started 5 sec after the injection and was

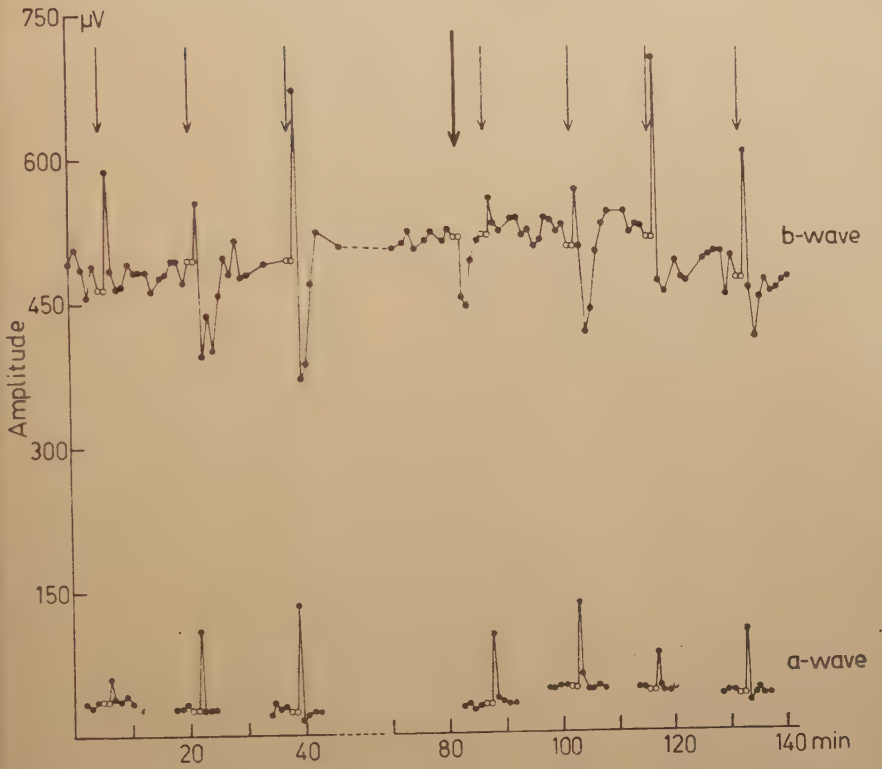


Fig. 5. – *Effect of nembutal on rabbit's ERG after injection of myanesin.*

Graph as Fig. 4 but the test dose of nembutal smaller, only 5 mg/kg i. a. Three injections of nembutal were made before the injection of myanesin, 30 mg/kg i. v. A pause of 45 min preceded the latter. Then followed four injections of nembutal (same dose) and the first ERG was recorded 5 sec after each injection. Stimulus intensity 96,000 lux. No variation of the a-wave after myanesin.

repeated every minute, sometimes up to 60 min afterwards. Strong stimuli were always used in these experiments. Neither by itself nor when preceded by eserine had acetylcholine any effect on the ERG.

In two animals nembutal (5 mg/kg i. a.) was given 1 min after an intravenous injection of eserine but in both cases the usual effect of the barbiturate was observed.

## Discussion

The plan of this work was to test the neural theory for explaining the potentiating effect of nembutal on the a- and b-wave of the ERG by using synaptic poisons or transmitters to modify the general neural "background" of the responding retina. While it remains an assumption that neural transmitters or synaptic poisons act on the neural inhibitory structures, postulated by Danis (6) and Wohlzogen (20) to be suppressed by nembutal and thereby to release the full ERG, it nevertheless seemed worth while making the experiments planned. It has now been shown that, unless there is a concomitant fall of blood pressure, the characteristic increase of the ERG after a small intra-arterial dose of nembutal is always obtained and but little, if at all, influenced by the poisons and transmitters used, myanesin, acetylcholine, eserine, DHE. While therefore these experiments do not exclude a neural theory for explaining the effect of nembutal on the ERG, they do suggest that synaptic transmission is unlikely to be involved. Electrotonic neural effects may well be present, e. g. after myanesin, which has a strong depressant action on synaptic transmission and, therefore, on any transmission theory, would have been expected to interfere with the postulated release of the structures generating the ERG.

## Summary

It is known from earlier work that nembutal strongly influences the size of the a- and b-wave of the electroretinogram, and an explanation in terms of release from tonic inhibition from nervous structures in the retina has been proposed.

This theory is subjected to a test making use of myanesin, dihydro-$\beta$-erythroidine, eserine and acetylcholine to alter internal retinal synaptic activity before testing with intra-arterial injections of nembutal in small doses.

It was found that none of these substances in doses which do influence synaptic activity in the spinal cord can prevent the potentiating effect of the nembutal injections on the electroretinogram.

Acknowledgements

The dihydro-β-erythroidine used in these experiments was made available by Merck and Co., U. S. A. The author is indebted to *Istituto Nazionale Assicurazione Infortuni sul lavoro* for a Travelling Fellowship.

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# A SUPRASPINAL CONTROL SYSTEM MONOSYNAPTICALLY CONNECTED WITH AN ASCENDING SPINAL PATHWAY

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## Introduction

This investigation is concerned with the supraspinal control of a pathway which ascends in the ventral quadrant of the spinal cord from the lumbar segments to the brain stem. Its neurones are characteristically activated by group II and III muscle afferents, skin and high threshold joint afferents of a bilateral, very wide, receptive field (15). Electrical stimulation of cutaneous and muscular hindlimb nerves elicits a train of impulses in these neurones. The excitation from the primary afferents is probably mediated by interneurones. In a previous paper an inhibitory supraspinal control of this pathway was described in some detail (5). This controlling system inhibits transmission of excitation from hindlimb nerves, presumably at an interneuronal level.

The present paper will show that this ascending pathway receives excitation from supraspinal centres. A descending tract in the contralateral ventral quadrant of the cord makes monosynaptic connections with the ascending pathway. The descending tract originates from cells in the brain stem. These cells may be activated from certain areas of the cortex of the anterior cerebellar lobe and thereby cause activation of the ascending spinal pathway. This activation may be inhibited from other areas of the anterior lobe cortex.

## Methods

The experiments were performed on unanaesthetized cats. The animals were operated on under ether anaesthesia and then decerebrated usually at a precollicular level when investigating effects from cerebellum. Flaxedil was given in experiments with stimulation of the brain stem.

The hindlimbs were denervated and some nerves mounted for stimulation. The right half of the spinal cord, except the sectioned dorsal column, was dissected for recording the discharge in ascending pathways. This dissection was usually performed at the Th8 segment and when not otherwise stated latency measurements refer to this recording position. In some experiments a laminectomy at the L1 segment permitted microelectrode recording from single ascending fibres which contributed to the mass discharge recorded from the more rostrally dissected spinal half.

In experiments for investigation of effects from the cerebellum the anterior lobe was exposed. The stimulation was performed with a wick electrode (cathode) touching the cerebellar cortex, the indifferent electrode being placed in the left temporal muscle. In experiments for stimulation of the brain stem the cerebellum was usually removed. The stimulation was performed with an electrolytically pointed needle electrode (cathode) mounted in a Horsley-Clarke instrument, the indifferent electrode being placed as in the experiments with cortical stimulation.

The electrical stimuli applied to the cerebellar cortex and brain stem were square pulses of 0.5 msec's duration. In all other cases the stimuli were condensor discharges with a half decay of about 50 $\mu$sec.

In experiments with needle electrode stimulation the brain stem was afterwards fixed in 10 per cent formalin. Serial sections 50 $\mu$ thick were cut with the freezing technique and photographed unstained through a microscope. The micrographs showed the needle tracks and were used for preparing maps as those shown in Fig. 9. A similar technique was used for control of lesions in the spinal cord.

For details concerning dissection of spinal cord and microelectrode technique see previous papers (9, 10).

## Results

The following description refers to experiments in which the *right* half of the spinal cord has been dissected for recording the discharge in the *right* ascending pathway. This discharge has been used as an indicator of the activity in the *left* descending tract originating from cells in the brain stem. With this method it has been possible to localize the cells of origin of the descending tract and study the connections between these cells and the cerebellar cortex. Microelectrode recording from single units in the *right* ventral quadrant has permitted identification of the ascending fibres responsible for the recorded mass discharge.

### I. – *The excitatory pathway.*

1. *Stimulation of the cerebellar cortex.* — As shown in the two lower records of Fig. 1 stimulation of the hamstring nerves elicited typical discharges in the dissected spinal half. The volley in Ib afferents of the left nerve (L. H.) monosynaptically excited ventral

spino-cerebellar tract neurones and thereby caused the first spike-like discharge (13). The corresponding discharge evoked from the right nerve (R. H.) was due to monosynaptic excitation of dorsal spino-cerebellar tract neurones by group I afferents (9, 10, 11). The late discharges with long duration were evoked in other tracts activated by group II and III muscle afferents (9, 10, 15). Records A-D show recordings from the dissected half on double shock stimulation of the cerebellar cortex. With the electrode in positions

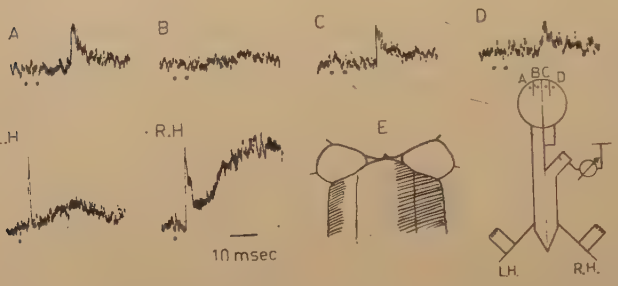


Fig. 1. – *Discharges evoked in the ascending spinal pathway on stimulation of the cortex of the anterior cerebellar lobe.*

The diagram (right) shows stimulating and recording arrangements. The circle represents the cerebellum with the anterior lobe divided in vermal and intermediate zones. A, B, C and D indicate positions of stimulating surface electrode (cathode). The indifferent electrode was placed in the left temporal muscle. Recording was performed from the dissected right half (except dorsal column) of the spinal cord at Th8. Stimulating electrodes were placed on left and right hamstring nerves (L. H. and R. H.). Records A, C and D show discharges evoked on double shock stimulation from corresponding positions of the anterior lobe. No discharge was evoked from position B. Double stimuli were used. The "excitatory areas" from which the ascending discharge could be elicited in this and other experiments are hatched in the diagram of the anterior cerebellar lobe (E). Lower records show the discharges evoked from the hamstring nerves. Dots mark stimulus artefacts. *Note.* The difference in amplitude of the late discharges on nerve stimulation is due to an asymmetric supraspinal control in the hemisectioned cord (cf. 5). In this and in the following figures about 10 haces were superimposed in each record.

A, C and D a discharge appeared. It should be noted, that the area of this discharge is considerably greater than that of the discharge evoked in the ventral spino-cerebellar tract. No discharge was elicited from position B.

In other experiments similar results were obtained. Double shock stimulation evoked a discharge appearing 8-13 msec after the second stimulus. This discharge will be denoted the *C-response*. It was obtained from two areas of the anterior lobe cortex. These will be denoted *excitatory areas* and are hatched in the diagram

shown in Fig. 1 *E*. The latency and magnitude of the discharge, and also the electrical threshold for evoking it, was the same for the two areas. The threshold was equal to, or slightly less than that causing abolishment of ipsilateral forelimb rigidity on repetitive stimulation. As a rule it was about 0.5 V or less. It was usually necessary to use double shock stimulation but in some experiments a single stimulus sufficed to evoke a discharge with a latency of 10 msec or more. In a few experiments, presumably due to a slight deterioration of the preparation, no discharge could be evoked from the cerebellar

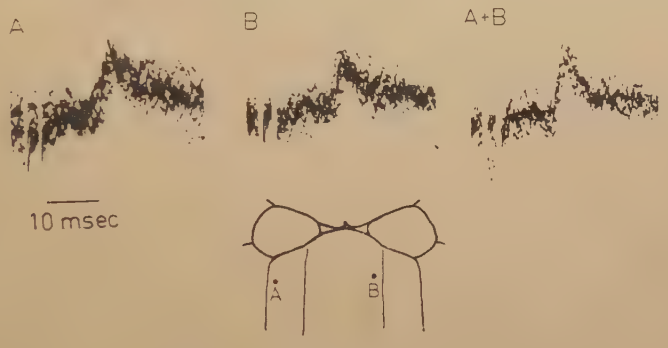


Fig. 2. – *Convergence of the pathways from the right and left excitatory areas of the cerebellar cortex.*

Stimulating and recording arrangements similar to those in Fig. 1. *A* : the discharge evoked from the left area; B: the discharge evoked from the right area. Double stimuli were used. The electrode positions are shown in the diagram of the anterior lobe. Right record (A + B) shows the discharge evoked by simultaneous stimulation of the two areas.

cortex despite the fact that decerebrate rigidity could be abolished on repetitive stimulation. Stimuli increased to several times the threshold for evoking a C-discharge from the excitatory areas were still ineffective when applied to the area of the cerebellar cortex, unhatched in Fig. 1 *E*.

Experiments as that illustrated in Fig. 2 suggest that the pathways from the two excitatory areas converge on to the same neurones. Stimulation of the left area at position A gave a discharge slightly larger than that evoked from position B of the right area. Simultaneous stimulation of the two areas evoked a discharge not appreciably larger than that evoked from position A alone. In this experiment only very slight inhibition was evoked from the exci-

tatory areas (cf. below), hence the effect is mainly or exclusively due to occlusion of the excitatory volleys.

There is evidence for complex pathways from the cerebellar cortex to the brain stem. The upper row of records in Fig. 3 shows an experiment in which a single stimulus sufficed to evoke the C-response. The latency was 14 msec. With two stimuli the latency in relation to the first stimulus was reduced to 13.1 and with three stimuli to 8.9 msec. Increasing the number of stimuli above three did not further reduce the latency (the two right records). These

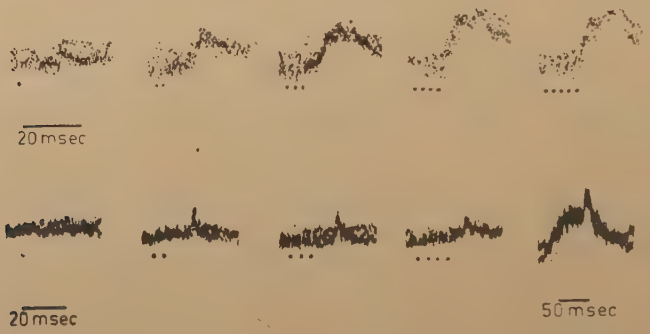


Fig. 3. – *Complex pathways from the excitatory areas of the anterior lobe as disclosed by repetitive stimulation.*

Stimulating and recording arrangements similar to those in Fig. 1. Upper and lower records from different experiments. Upper records show, with increased number of stimuli, applied on the border between vermis and the right intermediate cortex, a decrease of the latency due to facilitation by pathways having a short latency. Lower records show an increase of the latency due to inhibition (see text). The stimulating electrode was on the lateral part of the right vermis. Dots mark stimulus artefacts.

findings indicate that there are faster pathways from the cerebellar cortex than those yielding the usual response on single or double stimuli. The latency of the response as counted from the third stimulus was 5.9 msec. The discharge evoked by stimulation of the brain stem (see below) has a latency of about 4 msec. Hence it took only about 2 msec for the impulses to reach the brain stem when evoked in the cortex, whereas the corresponding latency on single shock stimulation was 10 msec.

In addition to the C-response, in a few experiments, a discharge with a latency of about 20 msec could be evoked on double shock stimulation. It was most marked from the unhatched area (Fig. 1 *E*) but could some-

times be discerned from adjacent areas following the C-response. Fig. 4 shows an experiment in which the 20 msec response was unusually large (B). It was also in this case smaller than the C-response (A).

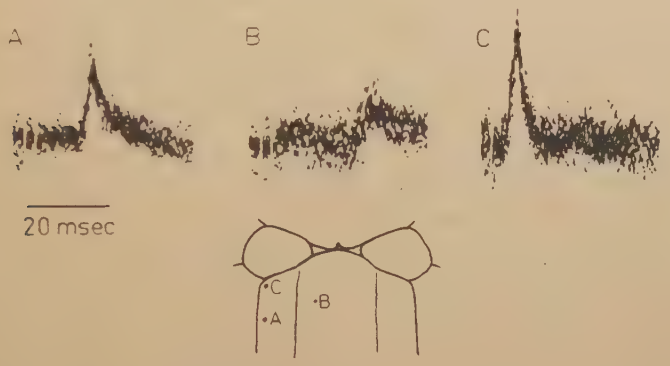


Fig. 4. – *Various types of discharges evoked in the ascending pathway by stimulation with a surface electrode on the cerebellar cortex.*

Stimulating and recording arrangements similar to those in Fig. 1. Records A, B and C show discharges elicited from the positions indicated on the diagram of the anterior lobe. The discharges in A and B are due to stimulation of cortical neurones whereas the discharge in C probably is due to stimulation of cerebellofugal fibres (see text). Double stimuli were needed to evoke the discharges in A and B, but not the discharge in C.

2. *Stimulation of the brain stem.* — Electrical stimulation of the brain stem would be expected to activate the fibres which convey the discharge from the excitatory areas of the cerebellar cortex to the spinal cord. A discharge with a latency of 3.5 to 4.5 msec appeared on stimulation of the brain stem. Increasing the stimulus strength did not evoke any later discharge. Hence the short latency discharge was due to activation of the fibres conveying the discharge from the cerebellar cortex. The former discharge will be denoted the *BS-response.*

The BS-response appeared at a low stimulus strength when the electrode was placed superficially in the region of the left lateral recess of the fourth ventricle. A typical discharge is shown in Fig. 5. The upper record was obtained with a slow and the middle with a fast sweep speed. The area of the BS-response was larger than the area of the discharge in the ventral spino-cerebellar tract evoked from the left hamstring nerve (L. H.). In the various experiments the BS-response had a duration of 5 to 12 msec.

When stimulating the left latero-ventral funicles of the cord a similar discharge is evoked at a shorter latency. This is illustrated in Fig. 6 with recording from the right spinal half at L2 and stimu-

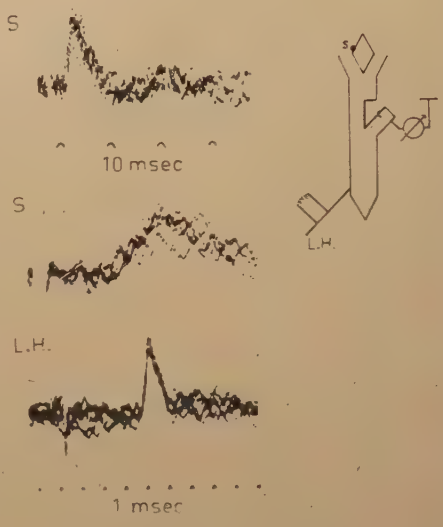


Fig. 5. – *Discharge evoked in the ascending pathway by stimulation in the brain stem.*

Stimulating and recording arrangements shown in the diagram. The cerebellum was ablated. S markes the position of the stimulating needle electrode (cathode). The indifferent electrode was in the left temporal muscle. Recording was performed from the right dissected spinal half (except dorsal column) at Th8. Upper and middle records, with different sweep speeds, show the discharge evoked by stimulation of the brain stem. Lower record shows the discharge in the ventral spino-cerebellar tract on stimulation of the left hamstring nerve (L. H.). Two lower records with the same time base.

lation of the left cord at Th 11 (record A) and C4 (record B). The distance between the stimulating electrodes was 14 cm and the latency difference of 1.4 msec gives a conduction velocity for the

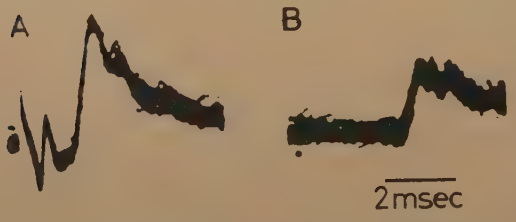


Fig. 6. – *Conduction velocity of descending pathway.*

Recording was made from the dissected right spinal half (except dorsal column) at L2 on stimulation of the lateroventral funicles in C4 (record B) and Th10 (record A). The distance between the stimulating electrodes was 14 cm and the distance from the stimulating cathode in Th10 to the dissected spinal half in L2 was 6.0 cm.

descending discharge of 100 m/sec. For the time being we may assume that the fibres stimulated in the left cord and those stimulated in the brain stem are the same although we are not able to exclude a propriospinal contribution. Measurements on axons conducting the ascending discharge in the right spinal half (cf. section 3) have revealed conduction velocities up to 100 m/sec.

It should now be considered if a latency of 3.5 msec from the brain stem and the latency in B, Fig. 6 is compatible with synaptic transmission from a descending to an ascending pathway. Alternatively these responses could be due to an axon reflex with one branch of an axon ascending in each spinal half. The distance from the brain stem to Th 8 where the right spinal half usually was dissected is 17-20 cm. If we allow 2 msec for descending conduction and 0.7 msec for synaptic transmission there would remain 0.8 msec for descending and ascending conduction below the level of the dissected spinal half, and the corresponding time in Fig. 6 would be 0.25 msec (observe dissection at L2). Hence there is time for one synaptic delay but an axon reflex with branching at a more caudal level cannot be excluded from these time measurement alone. Synaptic transmission of the effect is, however, proven by occurrence of spatial and temporal facilitation in this pathway as will now be described.

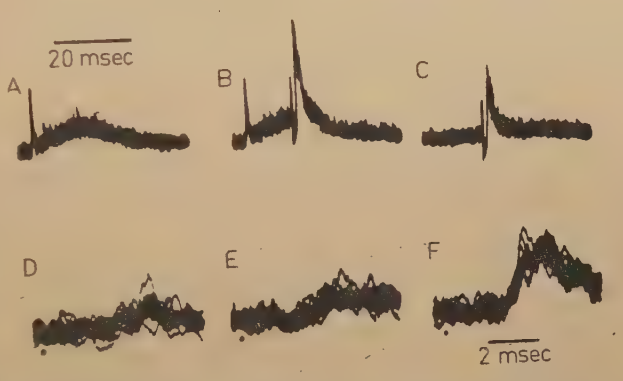


Fig. 7. - *Spatial facilitation of transmission in the descending-ascending spinal pathway.*

The upper and lower records are from different experiments, in both cases with recording from the right spinal half (except dorsal column) at L2. In A the left hamstring nerve was stimulated alone at 6 times threshold strength, which was sufficient to evoke a small late mass discharge following the synchronous discharge in the ventral spino-cerebellar tract. C shows the discharge from supramaximal stimulation of the left cord in Th10, and in B this discharge is facilitated by the conditioning volley from the left hamstring nerve.

In the experiment of D-F two filaments were dissected out of the left spinal cord quadrant at Th3 and mounted on separate pairs of stimulating electrodes. Separate supramaximal stimulation evoked the small discharges in Dand E and when stimulated simultaneously the facilitated discharge in F appeared.

In record A, Fig. 7, with recording from the right spinal half, the left hamstring nerve was stimulated at about 6 times threshold strength which was sufficient to evoke a small late mass discharge. This discharge is evoked in the ascending fibres activated by sti-

mulation of the brain stem or cerebellar cortex (see below, section I, 3). In record B this conditioning volley from the hamstring nerve considerably increased the test response evoked on stimulation of the left spinal cord which is shown unconditioned in record C. Spatial facilitation of a high degree is also disclosed by the experiment of records D-F. Two filaments of the left ventral quadrant were dissected and separate stimulation of them yielded the small responses in D and E, but when stimulated simultaneously the large discharge in F resulted.

Temporal facilitation in the pathway between the left and right cord is demonstrated in Fig. 8. The superimposed records in A

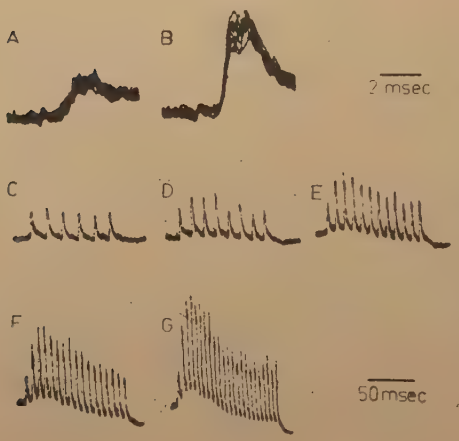


Fig. 8. – *Temporal facilitation of transmission in the descending-ascending spinal pathway.*

Recording from the right dissected spinal half (except dorsal column) at L2 and supramaximal stimulation of the dissected left ventral quadrant in Th3. Record A was taken before, record B after, tetanic stimulation during 30 sec at 250/sec. The growth of the response during tetanic stimulation at different frequencies is shown in C-G. Superimposed traces in A and B, single traces in C-G.

were obtained on stimulation of the left ventral quadrant in Th2. After repetetive stimulation at 250/sec for 20 sec, the considerably increased discharge in B was obtained post-tetanically. There was also a marked increase during repetetive stimulation particularly when higher frequencies are used, as is shown in records F and G, Fig. 8.

Since synaptic transmission of the effect has been proved, it is pertinent to consider the level in the cord, at which the relay occurs, in relation to the latency measurements discussed above. When recording from the upper lumbar segments it is clear that the rostral level at which the effects relays must be very close to the recording site. That this is the case was shown in experiments in which the cord was transected in the lumbar region at progressi-

vely more rostral levels or in which a median section was made in rostral direction from the sacral segments. With either type of lesion transmission of the early part of the discharge remained when the lesion was as close as one segment (about 10 mm) from the recording site. No corresponding lesions has been made with recording in Th8 but from the latency measurements it can be postulated that the upper level of the relay is in the lower thoracic segments.

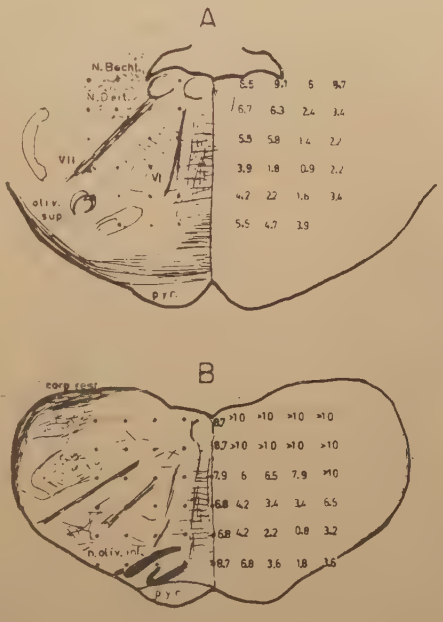


Fig. 9. – *Maps of brain stem thresholds.*

The left side of the brain stem was stimulated in order to locate the low threshold focus for direct stimulation of neurones with monosynaptic connections to the pathway ascending in the right spinal half. The cat was mounted in a Horsley-Clarke instrument and parallel tracks, 1 mm apart, were made at various transverse planes perpendicularly to the floor of the fourth ventricle. For each track the threshold for activation was measured at every mm and these points are shown to the left in the drawing. To the right are shown in corresponding sites the threshold values (volts) so obtained. The transverse plane of the tracks in A was 7.4 mm and in B 2 mm rostrally of obex.

In a series of experiments the brain stem was explored at various levels with a needle electrode mounted in a Horsley-Clarke instrument. The threshold values for evoking the BS-response at a transverse level 7,4 mm rostral to the obex are marked out on the map of Fig. 9 *A* and are representative of a typical experiment. Only the values obtained from the left half of the brain stem are shown. Stimulation of the right half yielded responses only when the strength was markedly increased so as to stimulate neurones in the left half. As shown in Fig. 9 *A* the lowest threshold values were found 3 mm laterally of the midline about 3 mm below the floor of the fourth ventricle. This is the region of the lateral reticular formation; it is clearly ventral to Deiters' nucleus. On systematic

transverse exploration at more proximal levels in the lower pons the threshold for evoking the BS-response gradually increased indicating that the responsible neurones did not extend very much cranially of the level in Fig. 9 *A*, Fig. 9 *B* shows the threshold values at a more caudal level 2 mm rostrally of obex.

It is known that in the spinal cord vestibulospinal and reticulospinal pathways descend in the ipsilateral ventral spinal cord quadrant. In our experiments a section of the dorsal half of the left lateral funicle did not decrease the BS-response recorded in the right spinal half. Hence the excitatory effect must be conveyed by fibres in the ventral quadrant. It is not from our experiments possible to decide if reticulospinal or vestibulospinal pathways are responsible because the low threshold values in Fig. 9 *A* and *B* may have been obtained on stimulation of axons and the vestibulospinal pathway is located in the relevant region of the brain stem.

The threshold values in Fig. 9 are for discharges with a latency of about 4 msec (the BS-response). From some places a discharge with a latency of about 5 msec (4.7 to 5.3 msec) could be elicited. Increased strength of stimulation resulted in its replacement by the BS-response. The 5 msec response was apparently due to activation of fibres presynaptic to those giving the response with a shorter latency. It appeared when the electrode was either rostral to the region giving the BS-response at a low threshold, or slightly lateral and caudal to that place.

The 5 msec response was regularly obtained at a fairly low stimulus strength with a surface electrode on the cerebellar cortex at the left rostral corner of the anterior lobe as shown in Fig. 4 *C*. The stimulus strength had to be increased above the strength necessary for eliciting the C-response. No corresponding discharge could be evoked from the right corner. With the electrode in the position where the 5 msec response could be evoked fibres in the cerebellar peduncles are stimulated at a fairly low stimulus strength (Lundberg and Oscarsson, unpublished.) Hence there is indication that discharges leave the cerebellum through the left cerebellar peduncles.

3. *Identification of the ascending fibres.* — The discharge evoked from the brain stem was not markedly reduced after a lesion in the dorsal half of the lateral funicle of the right side. Thus also the ascending pathway is in the ventral quadrant.

In several experiments axons in the ventral quadrant of the right side were explored with microelectrodes. Fig. 10 shows a unit which discharged a single impulse on stimulation of the brain stem (record E). It had a latency of 4.4 msec. Stimulation of various left and right hindlimb nerves evoked trains of impulses as illustrated for the left hamstring, the left superficial peroneal and the right tibial nerve in records A, B and C.

This unit belongs to a group of fibres described previously (15). These fibres ascend in the ventral quadrant of the cord and are acti-

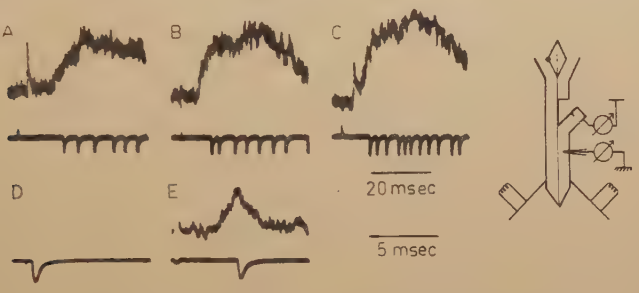


Fig. 10. – *Unit recording from ascending fibre activated from the brain stem.*

Microelectrode recording (lower trace) from fibre ascending in the right ventral quadrant of the spinal cord. The upper trace in A-C and E show the massdischarges in the dissected right spinal half (except dorsal column). The records in A, B and C were obtained on stimulation of the left hamstring, the left superficial peroneal and the right tibial nerve respectively. D is the antidromic activation of the fibre from the right spinal half and in E the left brain stem was stimulated with a needle electrode inserted through cerebellum.

vated from group II and III muscle afferents, skin and high threshold joint afferents. These units had a strong coupling with the descending fibres from the brain stem as shown by the fact that they could follow a high frequency train of impulses in the presynaptic fibres (up to 500 per sec). The spike had a minimum latency of 3.2 msec but appeared sometimes only after 5 to 10 msec. These values are consistent with the latency and duration of the BS-response as recorded from the dissected spinal half at the Th8 segment. The shorter latencies necessitate a monosynaptic coupling and fast descending fibres (100 m/sec). Possibly the coupling is monosynaptic also in the cases with a longer latency. The delay may be due to slower conduction velocities and more caudal levels of relay.

Stimulation of the excitatory areas of the cerebellar cortex evoked a discharge in the ascending units. For individual units the extension of the excitatory areas usually conformed well to the extension found on mass discharge recording.

It has been shown that some ventral spino-cerebellar tract neurones can be monosynaptically activated by stimulation of fibres in the contralateral ventral quadrant (14). The descending excitatory control of the ventral spino-cerebellar tract is possibly identical with the one explored in the present investigation. Weak inhibitory and excitatory effects on ventral spino-cerebellar tract neurones have been found on stimulation of the cerebellum (Lundberg and Oscarsson, unpublished).

Strong excitatory and inhibitory effects are exerted from cerebellum on another ascending spinal pathway with axons located mainly in the dorsal half of the lateral funicle. This pathway is excited by cutaneous afferents and by high threshold muscle afferents after a long delay and is not identical with any of the two pathways, excited by the flexion reflex afferents (12). The pattern of effects from cerebellum is different from those described in this paper and is possibly exerted by another controlling system.

II. – *Inhibition evoked by stimulation of the cerebellar cortex.*

Strong inhibition could be evoked from the unhatched field of Fig. 1 *E* lying between the two excitatory areas. This will be denoted the *inhibitory area*. In the experiment illustrated by Fig. 11 one electrode was placed on the left vermal cortex for conditioning the excitatory effect evoked by another electrode placed near the left lateral border of the anterior lobe. The conditioning electrode did not evoke any discharge on single or double shock stimulation (records A and B). The test response alone (evoked by double stimulation) is shown by records C and G. At certain intervals between the single conditioning and the two testing stimuli the response was completely abolished (D and E). The test response was unaffected when the conditioning stimulus followed 10 msec after the first testing stimulus (F). The inhibition was still considerable when it was applied 16 msec before the testing one.

The curve shows the inhibition measured as the reduction of amplitude of the discharge. The short latency of the inhibition is of special interest. The latency of the test discharge was 12 msec when counted from the first stimulus. Of these 12 msec roughly 4 were due to the conduction time from the brain stem to the recording place. The impulses causing the initial part of the discharge were consequently leaving the cerebellum about 8 msec after the first stimulus. A conditioning stimulus applied after 8 msec caused

an inhibition of 70 per cent. This shows that the inhibition, at least partly, was exerted in the nucleus of the brain stem or in the nucleus of the ascending spinal tract and not in cerebellum. In either case the inhibitory pathway from the cerebellar cortex to the brain stem must have a conduction time of the order of one or two msec.

Stimulation of the inhibitory area also reduced the 5 msec response evoked from the left rostral corner of the anterior lobe, which presumably is due to stimulation of the efferent fibres leaving

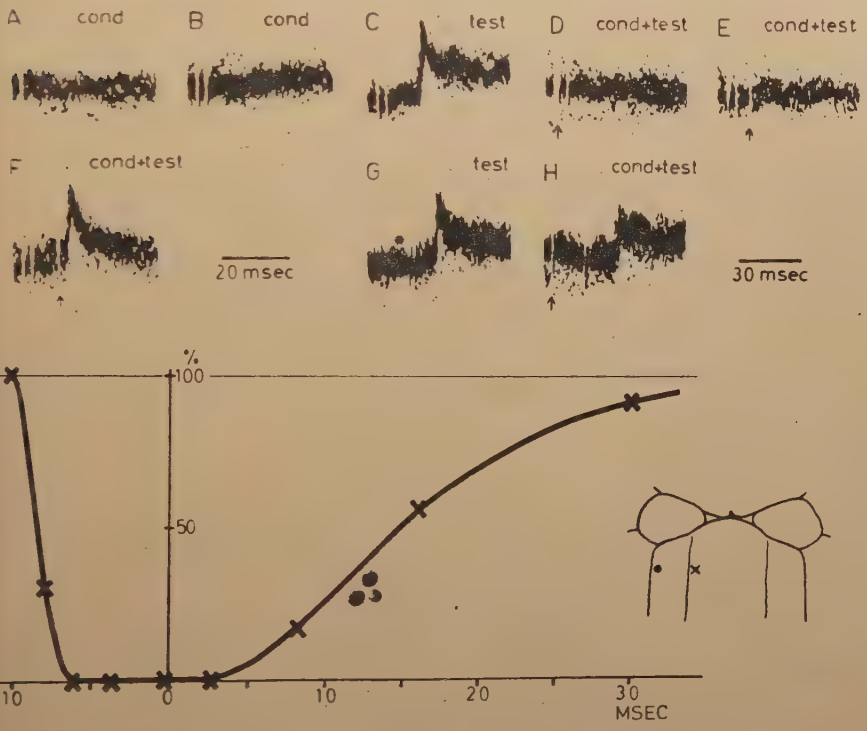


Fig. 11. – *Inhibition of the discharge elicited from the cortex.*

Stimulating and recording arrangements similar to those in Fig. 1. Positions of conditioning (×) and testing (·) electrodes shown in the diagram of the anterior lobe. The conditioning was performed with a single stimulus which did not evoke any discharge from this position (B). Neither did double shock stimulation evoke any discharge from this position (B). C and G show the unconditioned test response evoked by double stimuli. D, E, F and H show effects of conditioning at various intervals. Records G and H at a slower sweep speed. Arrows mark conditioning stimuli. The *curve* shows the inhibition measured as the decrease of amplitude of the test discharge. Abscissa: intervals between conditioning stimulus and first testing stimulus. Negative values indicate that the conditioning stimulus was applied after the testing stimulus.

the cerebellum. In the experiment illustrated in Fig. 12 a single stimulus to the inhibitory area reduced the test response to 50 per cent. The inhibition appeared when the conditioning and testing stimuli were about simultaneous. This is further indication of the high velocity of the inhibitory pathway.

Further experiments have shown that this inhibitory effect from cerebellum is exerted on the brain stem nucleus and not on the cells of the ascending spinal pathway. In Fig. 13 the test response in A was evoked on stimulation of the left rostral corner of the anterior lobe and the response in C on stimulation of the intact left

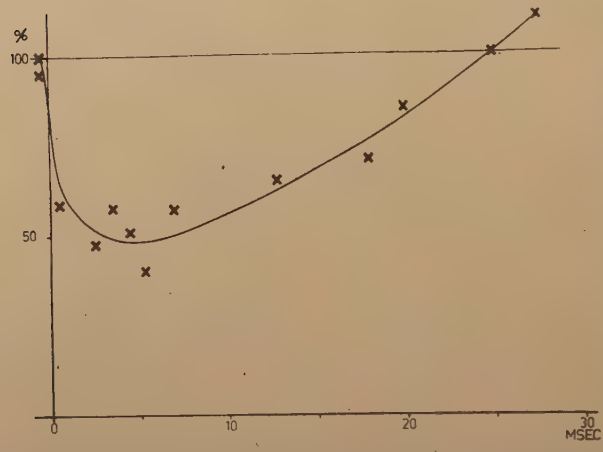


Fig. 12. – *Inhibition of 5 msec response evoked by stimulation at the left rostral corner of the anterior lobe.*

Stimulating and recording arrangements similar to those in Fig. 1 and 4. The test discharge was evoked by a single stimulus applied with a surface electrode at the left rostral corner of the anterior lobe. The single conditioning stimulus was applied to the inhibitory area. Ordinate: amplitude of conditioned test response in per cent of unconditioned one. Abscissa: interval between conditioning and testing stimuli. Negative values indica te that the conditioning stimulus came after the testing one.

latero-ventral funicles in the midthoracic region. A single conditioning stimulus to the left anterior vermis inhibits the former (B) but not the latter (D) test response.

The inhibition from the inhibitory area was regularly strong. Even in preparations lacking the excitatory effects from the cerebellar cortex (see above) the inhibition was marked as tested with the technique illustrated in Figs. 12 and 13. The inhibition appeared after single stimuli and had about the same threshold as the excitatory effect.

Axonal recording from units in the ascending pathway expectedly showed that the discharge evoked by stimulation of the excitatory areas was inhibited by stimulation of the inhibitory area.

Some of the units were spontaneously active and stimulation of the inhibitory area stopped the resting discharge.

Inhibitory effects were often encountered also on stimulation of the excitatory areas. The lower records in Fig. 3 were obtained from an experiment in which a single stimulus did not evoke any discharge. With double shock stimulation a discharge appeared with a latency (counted from the first stimulus) of 17.5 msec. With three stimuli the latency increased to 19.2 msec and with four to 20.1 msec. Hence there was an inhibitory effect with a shorter latency than the excitatory one. With a further increased number

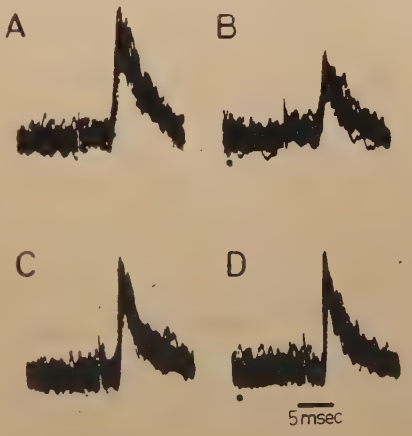


Fig. 13. – *Demonstration that the inhibition from cerebellum is exerted on the brain stem nucleus.*

Stimulating and recording arrangements as in Fig. 1 and 4. The test discharge in the upper records (shown alone in A) was evoked by a single stimulus applied at the left rostral corner of the anterior lobe. The test discharge in the lower records (shown alone in C) was evoked by submaximal stimulation of the intact left spinal half (dorsal column removed) in the mid thoracic region. In B and D a single conditioning stimulus was applied to the inhibitory area.

of stimuli the excitatory effect broke through as shown by the right record obtained with a slow sweep speed. The rebound which followed about 10 msec after the last stimulus suggests that part of the excitatory effect was concealed by inhibition.

In the experiment shown by Fig. 12 two stimuli were needed for evoking the C-response. Single stimuli applied to the excitatory areas resulted in a weak inhibition (less than 10 per cent) of the 5 msec response elicited from the left rostral corner of the anterior lobe. It had a maximum after 5 msec and could not be discerned after 10 msec.

## Discussion

The present investigation has given the outline of a control system which can ben influenced from the cortex of the anterior cerebellar lobe and activates a spinal pathway ascending from the

lumbar segments to the brain stem. This system has been investigated by thei ndirect method of recording from the ascending pathway.

The findings súggest the following organization of the control system influencing the *right* ascending pathway. Excitation of cortical neurones in certain areas of the anterior cerebellar lobe causes, through complex pathways, a cerebellofugal discharge in fibres which make monosynaptic connections with neurones in the brain stem. It has not been possible to decide if these neurones belong to vestibulospinal or reticulospinal pathways. The axons of the brain stem neurones descend in the left ventral quadrant of the cord and reach the lumbar segments to make monosynaptic connections with the ascending pathway.

The discharge evoked from the cerebellar cortex appeared on stimulation of two longitudinal zones denoted excitatory areas. Stimulation of a zone in between those, the inhibitory area, inhibited the discharge evoked from the excitatory areas. The organization of the cerebellar controlling system presently investigated indicates a subdivision of both the vermal and the intermediary cortex of the anterior lobe in two longitudinal zones, much as has been found for the organization of the zones influencing muscle tone.

The anterior lobe has been subdivided in one vermal part and one intermediate part (the lateral part is lacking in the cat) (3, 4, 6, 7). The corticonuclear fibres of the vermal cortex project mainly on to the fastigial nucleus and those of the intermediate cortex mainly on to the interposite nucleus (6). Physiological investigations have shown that the vermal part has two opposite actions on muscle tone. Facilitation of ipsilateral extensor tone is relayed through the rostromedial part of the fastigial nucleus and inhibition through the rostrolateral part (1, 2, 8). It is not known if the vermal cortex may be subdivided in two corresponding zonès. The intermediate cortex is functionally divided in one medial and one lateral strip (17). The medial strip is connected with the rostromedial part of the interposite nucleus and inhibits ipsilateral extensor tone (and causes ipsilateral active flexion) with opposite actions on the contralateral side. The lateral strip connected with the rostrolateral part of the interposite nucleus facilitates ipsilateral as well as contralateral extensor tone.

Our excitatory areas have the following extensions. The right area consists of the whole intermediate cortex and a lateral strip

of the vermal cortex. The left area consists of a lateral strip of the intermediate cortex (cf. Fig. 1 *E*). The inhibitory area is the zone between the two excitatory areas. When comparing this organization with the one influencing muscle tone it appears that our excitatory areas on the intermediate cortex facilitate extensor tone on the left side. Our excitatory area also includes the lateral part of the right hemivermis. The dominating effect on muscle tone when stimulating vermis is a bilateral inhibition of extensor tone but it has been demonstrated that on *supraliminal* stimulation of the vermal cortex the ipsilateral extensor tone is decreased and the contralateral increased (16). Hence it appears that the excitatory areas of the presently investigated system correspond to the cortical regions from which facilitation of the extensor tone on the left side can be evoked.

The pathways from the excitatory areas to the brain stem are complex. The latency for the most potent pathway was about 10 msec but on facilitation a discharge appeared with a latency of about 2 msec. Probably these pathways are relayed in the fastigial and interposite nuclei. The cerebellofugal fibres activate cells in the brain stem, presumably either in Deiters nucleus or in the lateral reticular formation. Axons from both these nuclei descend in the ipsilateral ventral quadrant of the cord (18, 19). Lesions in the spinal cord showed that the descending tract of the presently investigated system was in that part of the cord. The axons from Deiters nucleus reach the lumbar segments (18) whereas the available evidence indicates that fibres from the reticular nuclei end in cervical and thoracic segments (19). However, further experiments are needed to decide in which nucleus the cells of origin are situated.

The inhibition from the inhibitory area was regularly strong. This inhibition was, at least to a large extent, exerted at extracerebellar relays and it has been demonstrated that it affects the brain stem nucleus and not the spinal nucleus of the ascending tract. The inhibitory pathway from the cerebellar cortex to the brain stem had a latency of about one msec. It is tempting to connect this short latency with the Purkinje cells which project from the anterior lobe cortex beyond the roof nuclei to Deiters nucleus and possibly to other brain stem nuclei (6). The appearence of the inhibition after single stimuli and its persistence in preparations where the excitatory effect had disappeared is further evidence suggesting a pathway with a minimum number of synapses.

The functional significance of the control system here investigated is unknown as is indeed the role and the termination of the pathway itself. It is noteworthy that the hindlimb afferents exciting the ascending pathway are those evoking the ipsilateral flexor reflex while supraspinal excitation of the pathway is provided from cerebellar cortical areas which facilitate extensor tone as described above. It is as yet not possible to state that these two functions of the anterior cerebellum are carried out by the same neuronal systems. For this purpose further investigations are needed to disclose the excitatory pathways from the cerebellar cortex to the brain stem nucleus giving rise to the descending tract.

It is also of interest that transmission from hindlimb nerves to this ascending pathway is controlled by another supraspinal system (5). This control is inhibitory and is tonically active in the decerebrate preparation to a degree that transmission from the nerves may be completely suppressed. The responsible centres are located in the brain stem and the tonic control is uninfluenced by stimulation of the cerebellum leading to complete collapse of decerebrate rigidity and also by ablation of cerebellum and the vestibular nuclei. Presumably the inhibitory effect is exerted on the interneurones mediating excitation from the nerves and would not interfer with the monosynaptic transmission investigated in the present paper.

## Summary

This investigation, performed on unanaesthetized decerebrate cats, has been concerned with a supraspinal control system which can be influenced from the cortex of the anterior cerebellar lobe and excites a pathway ascending in the contralateral ventral quadrant of the spinal cord from the lumbar segments to the brain stem. The ascending pathway is characteristically activated by group II and III muscle afferents, skin and high threshold joint afferents of a bilateral, very wide, receptive field.

The control system influencing the *right* ascending pathway is organized as follows. Stimulation of cortical neurones in certain areas (the excitatory areas) of the anterior cerebellar lobe causes, through complex pathways, a discharge in cerebellofugal fibers. These fibres make monosynaptic connections in the brain stem with the cells of a descending pathway (undecided if vestibulospinal or

reticulospinal). The axons of this pathway descend in the left ventral quadrant of the cord and reach the lower thoracic and lumbar segments to make monosynaptic connections with the ascending pathway. The fastest of these axons had a conduction velocity of 100 m/sec.

There are two excitatory cortical areas of cerebellum. The right area consists of the right intermediate cortex and a lateral strip of the right vermal cortex. The left area consists of a lateral strip of the left intermediate cortex. The discharges elicited in the ascending pathway from the two areas were similar. Double shock stimulation of the cerebellar cortex was usually needed for evoking them. The conduction time from the cerebellar cortex to the brain stem varied between 6 and 9 msec for the initial part of the discharge.

The discharge evoked in the ascending spinal pathway on stimulation of the excitatory areas could be completely inhibited by stimulation of the inhibitory area which consisted of the cerebellar cortex between the two excitatory areas. The inhibition was exerted in the brain stem nucleus. The inhibition appeared on single stimuli and had a conduction time from the cerebellar cortex to the brain stem of about one msec.

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# ON THE ROLE OF THE EXTRALEMNISCAL PATHWAYS IN THE EEG AROUSAL REACTION ELICITED BY RETICULAR STIMULATION [1]

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## Introduction

The EEG arousal elicited by electrical stimulation of the reticular formation of the lower brain stem (8) is not abolished by midbrain transection of the classical lemniscal pathways (8). The activation of the electrocorticogram has been attributed to ascending reticular relays (8), which have been the object of several anatomical and physiological investigations in the last ten years (4, 6, 12). Anatomical data suggest that extralemniscal pathways, arising from spinal cord and sensory trigeminal nuclei, may be co-stimulated with the ascending reticular system. This paper is concerned with the possible role of these through fibers in the mediation of the EEG effects elicited by reticular stimulation.

About sixty years ago Wallenberg (14) destroyed the trigeminal sensory nucleus in the rabbit and found with Marchi's method a bundle of ascending extralemniscal fibers lying in the dorsolateral part of the brain stem at a transcollicular level. Also Quensel (11) described long paraventricular pathways near the dorsolateral border of the brain stem reticular formation. In 1954 Russel (13) published

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his anatomical findings about parareticular ascending pathways in the brain stem and concluded that Wallenberg's trigemino-thalamic tract might represent a " lateral reticulo-thalamic system of connections ". Nauta and Kuypers (10) came to the same conclusion, since degeneration of Wallenberg's tract occurred in their experience only when the lesion had encroached upon the reticular zone which separates the main sensory from the motor trigeminal nucleus. They reported, however, that lesions confined to the descending trigeminal nucleus were followed in the cat by " extremely profuse and widespread fiber degeneration dispersed over the reticular formation bilaterally ". The pontine and mesencephalic course of this extralemniscal trigeminal fiber system was reported (10) to be much similar to that of the diffuse tegmental conduction pathway arising in the spinal cord.

In the present experiments an attempt was made to investigate whether *bulbar reticular stimulation still yields EEG arousal when all the long pathways (lemniscal, extralemniscal, pyramidal) are interrupted at a rostral level.*

## Methods

The experiments were performed on cats. Under ether anesthesia the skull was opened just behind the level of the bony tentorium and the lateral parts of the brain stem were cut electrolytically with the aid of a stereotaxic instrument at the posterior border of the inferior colliculi, sparing the reticular formation 1 to 3 mm from the midline on both sides. This level was chosen following a suggestion of Dr. Nauta (9), who pointed out that a complete section of the lateral lemniscal and of the extralemniscal pathways was likely to be achieved at the level of the inferior colliculus with only partial sacrifice of ascending reticular bundles. In order to avoid the bony tentorium, the coagulating electrode (stainless steel, monopolar) was introduced with an inclination of 30° to the vertical through the cerebellum. In a first group of cats the lesion was incomplete, in a second group the lesion was extended ventrally to the basilar bone. The section of the pyramidal tract and medial lemniscus was performed from a ventral, retropharyngeal approach with a fine knife, under visual control. The level of this latter section was 1 mm caudal to the inferior border of the pons (Fig. 1).

The bulbar reticular formation was stimulated through a concentric needle electrode at P 13 level of the stereotaxic coordinates. Rectangular pulses 1 msec in duration, 300/sec, 2-5 V were applied with a Grass P 4 stimulator. The physiological test of the localization of the electrode tip in the medial bulbar reticular formation was the well known inhibition of the knee jerk (7).

Six electrodes screwed into the skull above the premotor, sensorimotor and occipital areas picked up, through intact dura, the cortical electrical activity, which was registered with a 6-channel inkwriter Grass electroencephalograph.

The experiment started one hour after termination of the ether anesthesia. The extent of the lesions and the localization of the stimulating electrode were verified histologically in serial slides.

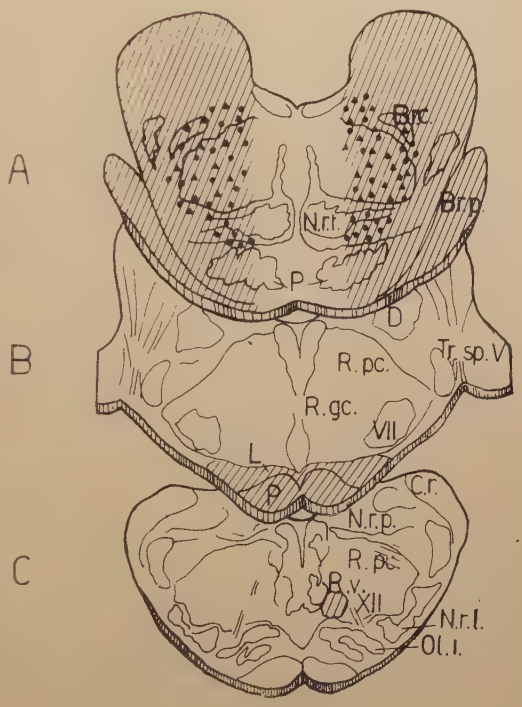


Fig. 1. – *Experimental arrangement.*

Drawings of transverse sections of the cat's brain stem made at different levels. *A*: the hatched areas indicate the extension of the electrolytic lesion. At the level of the inferior colliculus the lesion has transected a large lateral tegmental region bilaterally, sparing a paramedian zone approximately 2 mm wide on both sides. Dots represent the trigeminal extralemniscal fibers; small triangles the spinal extralemniscal fibers (9). *B*: at the level of the nucleus facialis the hatched areas represent the interruption by knife of the pyramidal tract and of the medial lemniscus. *C*: at the level of the inferior olive the hatched area represents the electrolytic lesion made in the medial bulbo-reticular formation with the inner tip of the stimulating concentric electrode in position.

*Abbreviations*: Br.c.: brachium conjunctivum; Br.p.: brachium pontis; C. r.: restiform body; D.: Deiters' lateral vestibular nucleus; L.: lemniscal pathway; N. r. l.: lateral reticular nucleus (nucleus of lateral funiculus); N r. p.: nucleus reticularis paramedianus of Brodal; N. r. t.: nucleus reticularis tegmenti pontis; Ol. i.: inferior olive; *P*: in *A* = pontine nuclei, in *B* = pyramidal tract; R. gc.: nucleus reticularis gigantocellularis of Meessen and Olszewski; R. v.: nucleus reticularis ventralis of Meessen and Olszewski; Tr. sp. V: spinal tract of trigeminus; VII: nucleus facialis; XII: hypoglossal fibers.

## Results

We shall be concerned here only with those experiments in which bilaterally complete interruption of both lemniscal and extralemniscal pathways and of the pyramidal tract had been obtained. One to several hours after the operation these cats presented the typical EEG synchronization that characterizes the *cerveau*

*isolé* animal when the width of the paramedian strip spared by the lesion extended 2 mm on each side. EEG arousal was easily elicited in these animals with the usual parameters (2-3 V) of electrical stimulation (Fig. 2). The persistence of the EEG response following

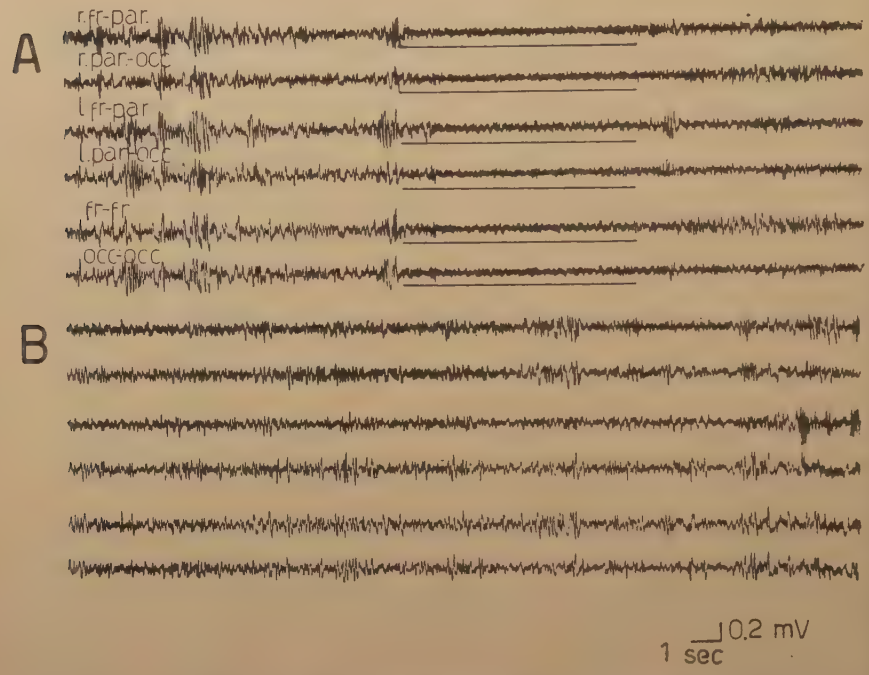


Fig. 2. – *Effect of reticular stimulation on EEG patterns of a cat after electrolytic lesion at pontine level and section of pyramidal and medial lemniscal pathways at upper bulbar level.*

*A*: EEG recordings from right (r. fr. - par.) and left frontoparietal (l.fr.-par.), right (r.par.-occ.) and left parieto-occipital (l.par.-occ.), fronto-frontal (fr.-fr.) and occipito-occipital (occ.-occ.) leads after lateral electrolytic lesion at pontine level and interruption of pyramidal tract and lemniscal pathway at upper bulbar level (see Fig. 1). Stimulating electrode in medial bulboreticular formation. Reticular stimulation (300/sec; 1 msec; 2. 5 V) produces clear cut arousal reaction (black line). *B*: Recovery of EEG synchronization at the end of reticular stimulation (30 sec after *A*).

transection of the pyramidal tract and medial lemniscus at a more rostral level effectively disposes of the objection that spread of current, or stimulation of pyramidal tract collaterals ending in the reticular formation might yield EEG arousal through extrareticular, antidromic or orthodromic paths. Whenever the incision was deep-

ened so that the upper medullary lesion encroached upon the overlying bulbar reticular formation, the arousal effect disappeared.

We have mapped the reticular formation at lower bulbar levels in order to study the topographycal relations between the points yielding inhibition of knee jerk and those producing an arousal reaction. The inhibitory effects were obtained by stimulating the ventromedial reticular formation only, in confirmation of the well known results of Magoun and Rhines (7), while the area yielding an EEG arousal reaction extended more laterally and occasionally more caudally, also in confirmation of previous findings (8). Stimulation parameters were the same for both effects.

## Discussion

Our experiments show that the EEG arousal produced by reticular stimulation is mediated by fibers arising in the reticular formation itself. Co-stimulation of through fibers belonging to extralemniscal sensory paths may possibly contribute to, but is by no means essential for, the appearance of the electrocortical response.

Lindsley, Bowden and Magoun (5) had previously shown that selective destruction of all "classical" sensory pathways, carried out at midbrain level, did not diminish the EEG activation patterns of the *encéphale isolé*, while destruction of large areas of the midbrain tegmentum abolished EEG activation and precipitated recurring bursts of spindles. These experiments were the first demonstration that the EEG activating discharge ascending along the inner core of the brain stem is tonic in nature. This ascending discharge could not be mediated, in the experiment of Lindsley, Bowden and Magoun (5), by the diffuse tegmental system of ascending spinal fibers studied by Nauta and Kuypers (10), since the spinal cord had been transected at C 1. It might still be maintained, however, that the EEG sleep patterns occurring after midbrain tegmental lesions could be due to severance of through fibers arising from sensory trigeminal nuclei and ascending as extra-lemniscal pathways through the tegmentum (10). This objection was, however, likewise disproved by the observations of Batini, Moruzzi, Palestini, Rossi and Zanchetti (2, 3) and Batini, Magni, Palestini, Rossi and Zanchetti (1), who showed that persistent patterns of activation characterize the EEG of the midpontine pretrigeminal preparation.

On the whole both lines of evidence – the one obtained from brain stem lesions and our present experiment of electrical stimulation – support the hypothesis (8) that the activating influence of the brain stem is mainly mediated by an ascending reticular system.

## Summary

The bulbar reticular formation was electrically stimulated in cats, in which the lemniscal and extralemniscal sensory pathways and the pyramidal tract had been bilaterally and totally severed at more rostral levels. Since the EEG arousal was obtained with the usual parameters of electrical stimulation, the hypothesis that the "reticular" arousal is due to co-stimulation of extralemniscal (but not reticular) pathways ascending diffusely along the inner core of the brain stem may be regarded as disproved.

## Acknowledgements

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# INFLUENCE DU LOBE ANTÉRIEUR DU CERVELET SUR L'ACTIVITÉ TONIQUE ET RESPIRATOIRE DES MUSCLES INTERCOSTAUX

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## Introduction

On a longtemps cru que le contrôle exercé par le cervelet sur les voies motrices se limitait au maintien de la posture et à la coordination des mouvements et c'est en 1938 seulement que la régulation des fonctions végétatives par le cervelet, bien que déjà entrevue par plusieurs auteurs (3, 2, 15, 9), fut démontrée par Moruzzi (voir 10). Ce dernier observa en effet que la stimulation faradique du lobe antérieur du cervelet, chez le chat décérébré, inhibait non seulement le tonus de posture, mais également certains réflexes respiratoires: très nets au cours d'une hyperpnée réflexe, les effets respiratoires de cette stimulation étaient au contraire insignifiants chez le chat décérébré respirant normalement.

Pour notre part nous nous sommes demandé si, tout en ne modifiant pas le débit respiratoire, cette stimulation cérébelleuse n'agissait pas tout de même sur les muscles du thorax. Massion, Meulders et Colle (8) ont montré en effet que les réflexes posturaux pouvaient influencer la musculature thoracique sans modifier le débit respiratoire, et dès lors il était possible que la stimulation du lobe antérieur du cervelet agisse sur ces muscles de la même manière que les réflexes posturaux.

Nous nous sommes donc proposé de rechercher si, chez le chat décérébré et en eupnée, la stimulation du lobe antérieur du cervelet n'influençait pas l'activité des muscles respiratoires, et pour ce faire, nous avons observé les réactions respiratoires en enregistrant, d'une

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part le rythme et le débit respiratoires au niveau de la canule trachéale, et d'autre part l'activité électromyographique des muscles de la cage thoracique.

## Méthodes

Nous avons pratiqué la décérébration des chats par une section du tronc cérébral au niveau pré- ou intercolliculaire, sous anesthésie à l'éther. Afin d'éviter les risques d'hémorragie, nous avons momentanément interrompu la circulation carotidienne pour procéder à la section de l'axe nerveux.

L'excitation du cervelet a été effectuée au moyen d'électrodes terminées par des boules d'argent de 1 mm de diamètre, la distance interélectrodes ne dépassant pas 2 mm. L'appareil d'excitation utilisé fournit des courants rectangulaires de durée et d'intensité réglables. La plupart des résultats ont été obtenus au moyen de courants de 1 msec et d'une fréquence de 300 par seconde.

Dans une première série d'expériences nous avons enregistré le débit respiratoire de l'animal; celui-ci respirant librement, nous nous sommes servis d'un montage électronique comprenant une lampe du type transducteur dont l'anode est reliée mécaniquement à une lamelle placée dans la canule trachéale et mobilisée par l'air tour à tour inspiré et expiré. On peut ainsi enregistrer le débit respiratoire à chaque instant.

Dans une seconde série d'expériences, nous avons enregistré l'activité électrique des muscles respiratoires et pour cela, exploré successivement, au moyen d'électrodes concentriques reliées à un appareil d'électromyographie, tous les espaces thoraciques dans leur portion dorsale et ventrale, ainsi que le muscle diaphragme.

## Résultats

1. *Influence de la stimulation du lobe antérieur du cervelet sur la fréquence et le débit respiratoire.* — Dans ces expériences, effectuées sur chats décérébrés, nous avons étudié l'influence de la stimulation du cortex du lobe antérieur cérébelleux sur la fréquence et le débit respiratoire, enregistrés au niveau de la canule trachéale, et à cette fin excité au moyen d'électrodes bipolaires, les lobules III, IV et V du lobe antérieur. Les lobules I et II, inaccessibles à cause de leur situation anatomique, n'ont pu être étudiés.

Avant de rechercher tout nouveau résultat, nous avons examiné l'état de notre préparation en vérifiant si nous retrouvions les effets déjà connus de la stimulation du cervelet sur le tonus postural: inhibition par la stimulation cérébelleuse de la rigidité posturale, chez le chat décérébré, décrite par Löwenthal et Horsley (6) et Sherrington (14), de plus, inhibition ipsilatérale à la partie du cortex cérébelleux excitée, et atteignant les segments musculaires correspondant à cette aire, ainsi que le démontrèrent Hampson, Harrison et Woolsey (4 et 5).

De fait, nous retrouvâmes la somatotopie décrite par ces derniers: la stimulation du lobule VI atteint les muscles du cou; celle des lobules IV et V et celle du lobule III, influencent respectivement les muscles des membres antérieurs et postérieurs. L'inhibition ispilatérale du tonus postural est la plus nette lorsqu'on excite le vermis cérébelleux, mais nous l'avons également obtenue, quoique souvent accompagnée d'une flexion active (13), en stimulant la région paravermienne (partie médiane du lobe intermédiaire). Enfin, nous avons fréquemment observé une augmentation du tonus postural contralatéral au cortex cérébelleux excité, ainsi que Bremer (1) l'avait signalé.

En dehors de ces effets classiques de la stimulation cérébelleuse sur le tonus postural, nous avons alors recherché si cette stimulation influençait également le rythme et le débit respiratoire et constaté que la stimulation cérébelleuse n'a que des effets inconstants sur la respiration.

Nous avons donc admis que la stimulation du lobe antérieur et du lobule VI du cervelet n'influençait guère le rythme ou le débit respiratoire, résultat comparable à celui de Moruzzi (10).

2. *Influence de la stimulation du lobe antérieur du cervelet sur le tonus postural de la cage thoracique.* — Pour compléter le résultat précédent, nous nous sommes proposé d'enregistrer l'activité électromyographique des muscles respiratoires et de rechercher, grâce à cette technique, qui permet d'explorer séparément les muscles intercostaux et le muscle diaphragme, l'influence de la stimulation cérébelleuse sur cette activité.

Nous avons déjà décrit, dans un travail précédent (8), l'activité électromyographique des muscles respiratoires et nous n'en rappellerons ici que brièvement les conclusions: nous avons remarqué que les muscles intercostaux internes ou externes, aussi bien ventraux que dorsaux, présentent, chez le chat décérébré, non seulement une activité respiratoire, mais également une activité tonique qui peut être continue ou renforcée pendant l'inspiration ou l'expiration. Celle-ci donne lieu à la rigidité posturale de la cage thoracique observée chez l'animal décérébré. Ce tonus postural des muscles intercostaux est influencé par les réflexes posturaux du cou et du labyrinthe, comme celui des muscles éxtenseurs des membres (8). Le diaphragme, par contre, présente une activité uniquement inspiratoire et n'est pas influencé par les réflexes posturaux.

Dans les expériences qui nous occupent ici, nous avons recherché l'influence de la stimulation cérébelleuse sur le tonus postural des muscles intercostaux et exploré, comme dans le chapitre précédent, les lobules cérébelleux III, IV, V et VI de Larsell.

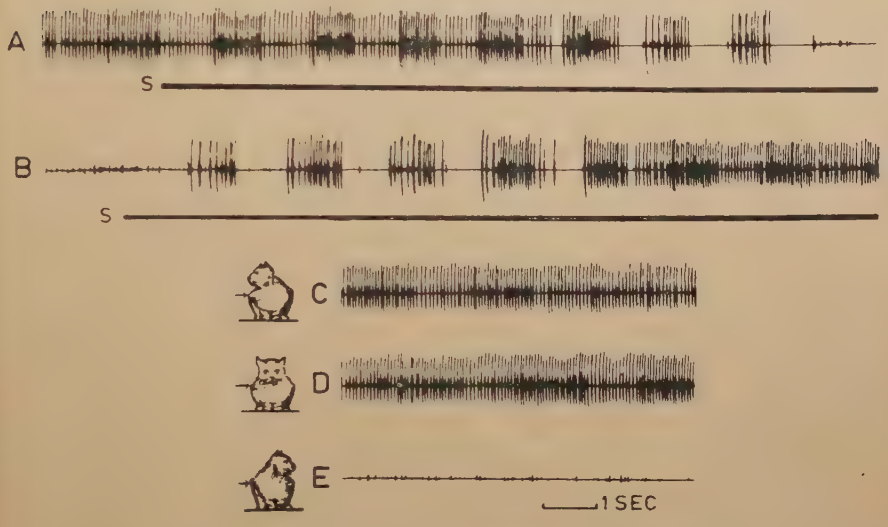


Fig. 1. – *Influence de la stimulation du lobe antérieur du cervelet et des réflexes toniques du cou sur l'activité myographique d'une même unité motrice intercostale droite, dont l'activité tonique est renforcée pendant l'inspiration.*

Chat décérébré. Sur les schémas de chat, la flèche indique le côté de l'enregistrement.

*A*: stimulation du lobe *V* ipsilatéral en *S*. Notez l'inhibition de l'activité tonique de l'unité motrice qui se manifeste dès le début de la stimulation. L'activité inspiratoire de cette unité motrice finit également par disparaître.

*B*: effet facilitateur de la stimulation du lobule *V* contralatéral en *S*. Pendant la stimulation, l'activité de l'unité motrice réapparaît: elle est d'abord inspiratoire et devient ensuite tonique avec renforcement inspiratoire.

*C*, *D* et *E*: influence des réflexes toniques du cou sur l'activité de la même unité motrice. En *C*, après rotation contralatérale, l'unité cesse toute activité.

En enregistrant l'activité électromyographique de la paroi thoracique, on observe que l'excitation vermienne ou paravermienne des lobules IV et V, zone somatotopique du membre antérieur et de l'épaule, inhibe l'activité tonique des muscles intercostaux du même côté. Ce résultat est obtenu par un courant électrique de 0,5 à 1 V et d'une fréquence de 300 chocs par seconde. La Fig. 1 *A* montre

comment l'activité tonique spontanée ipsilatérale d'une unité motrice intercostale disparaît totalement par la stimulation du cervelet, pour réapparaître, après la fin de celle-ci, avec une intensité accrue.

Cette inhibition, et l'intensification de l'activité tonique intercostale, qui suit la fin de l'excitation, sont semblables aux réactions provoquées dans les muscles extenseurs par la stimulation du cervelet. On voit, sur la Fig. 2 *A*, l'analogie des réponses toniques d'un muscle extenseur (le muscle triceps de la patte antérieure) et d'un muscle intercostal à la stimulation cérébelleuse. Notons enfin que pendant cette stimulation, l'activité tonique intercostale peut être temporairement rythmée par la respiration, avant de disparaître complètement (Fig. 1 *A*).

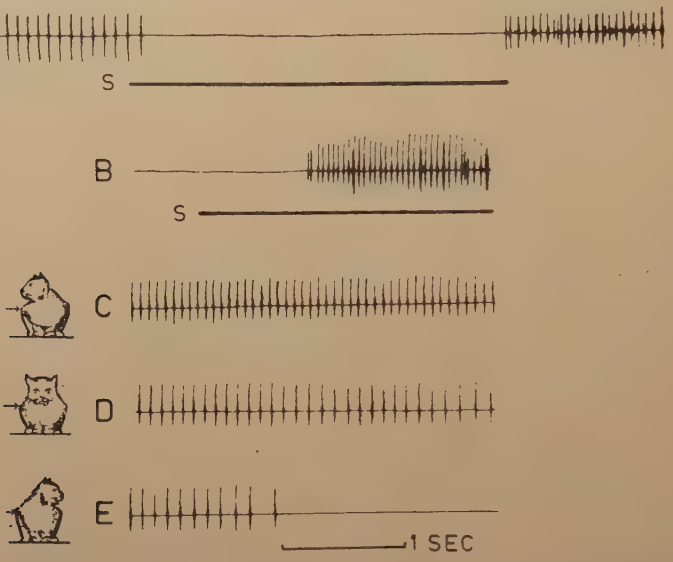


Fig. 2. – *Influence de la stimulation du lobe antérieur du cervelet et des réflexes toniques du cou sur l'activité myographique d'une même unité motrice tonique du triceps droit.*

Chat décérébré. Sur les schémas de chat, la flèche indique le côté de l'enregistrement.

*A*: stimulation du lobule *V* ipsilatéral en *S*. Notez l'inhibition de l'unité motrice pendant la stimulation et la facilitation postexcitatoire.

*B*: stimulation du lobule *V* contralatéral en *S*. L'unité motrice reprend son activité sous l'effet facilitateur de la stimulation.

*C*, *D* et *E*: influence des réflexes toniques du cou sur l'activité de l'unité motrice tricipitale droite. En *C*, après rotation ipsilatérale de la tête, l'activité est la plus intense. En *E*, après rotation contralatérale, l'activité se réduit, puis disparaît.

Si, par contre, nous excitons le même lobule, mais contralatéralement, ce n'est pas une inhibition, cette fois, mais une intensification de l'activité tonique musculaire que nous obtenons. La stimulation contralatérale du cervelet fait apparaître une activité respiratoire d'abord, puis tonique, dans une unité motrice musculaire précédemment inactive (Fig. 1 *B*). A titre de comparaison, nous avons représenté sur la Fig. 2 *B*, l'apparition d'activité tonique dans le muscle triceps du membre antérieur, à la suite d'une stimulation contralatérale du cervelet.

Il ressort donc que la stimulation électrique des lobules IV et V du cervelet, région somatotopique du membre antérieur et de l'épaule, inhibe l'activité tonique des muscles intercostaux ipsilatéraux et facilite celle des muscles intercostaux contralatéraux.

Or différents arguments nous permettent de considérer cette activité comme une véritable activité posturale: le fait qu'elle soit exagérée par la décérébration, l'influence exercée sur elle par les réflexes posturaux du cou et du labyrinthe – ces deux points ont été établis dans nos travaux précédents (8) –, enfin, le fait que la stimulation cérébelleuse et les réflexes cervicaux influencent de la même manière l'activité tonique intercostale et celle des muscles extenseurs des membres (Figs. 1, 2 *A-E*).

On peut donc conclure que le cervelet, agissant sur l'activité tonique des muscles intercostaux, exerce bien une action sur le tonus postural de la cage thoracique.

3. *Influence de la stimulation du lobe antérieur du cervelet sur l'activité respiratoire des muscles de la cage thoracique.* — Nous nous intéressons ici aux effets de la stimulation cérébelleuse sur l'activité proprement respiratoire des muscles de la cage thoracique: muscles intercostaux et muscle diaphragme.

Une première série d'expériences nous a permis de constater que la stimulation des lobules IV et V, dans la région vermienne et paravermienne, n'influence pas seulement l'activité des muscles intercostaux lorsqu'elle est continue, c'est-à-dire tonique, mais également lorsqu'elle a un rythme respiratoire: lors d'une stimulation ipsilatérale du cervelet, on voit la fréquence des potentiels d'action, au cours d'une volée inspiratoire ou expiratoire, diminuer et même finir par disparaître complètement (Fig. 3 *A*). Par contre, dès la fin de la stimulation, se manifeste une intensification comparable à celle que nous avons observée pour l'activité tonique des interco-

staux ou des extenseurs des membres: l'activité de l'unité motrice musculaire réapparaît sous forme de volées à systématisation respiratoire ou d'activité tonique intense (Fig. 3 *A*); cette dernière perd son intensité après quelques secondes pour se laisser à nouveau rythmer par la respiration.

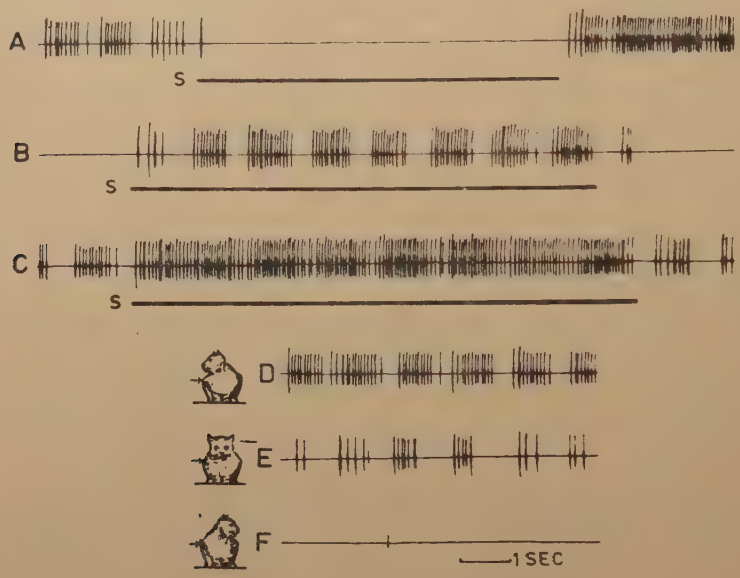


Fig. 3. – *Influence de la stimulation du lobe antérieur du cervelet et des réflexes toniques du cou sur l'activité myographique d'une même unité motrice intercostale droite, expiratoire.*

Chat décérébré. Sur les schémas de chat, la flèche indique le côté de l'enregistrement.

*A*: stimulation du lobule *V* ipsilatérale en *S*; notez l'inhibition de l'unité motrice pendant la stimulation et la facilitation postexcitatoire de cette unité qui devient tonique.

*B* et *C*: effet facilitateur de la stimulation du lobule *V* contralatérale en *S*. La stimulation en *B* fait réapparaître l'activité de l'unité motrice et en *C* transforme l'activité expiratoire en activité tonique.

*D*, *E* et *F*: influence des réflexes toniques du cou sur l'activité de la même unité motrice. En *D*, après rotation ipsilatérale de la tête, l'activité est la plus intense. En *E*, après rotation contralatérale l'activité de l'unité motrice disparait.

Par contre, la stimulation contralatérale des mêmes lobules provoque, non pas une inhibition, mais une intensification de l'activité musculaire respiratoire, qui peut se traduire de deux manières différentes: si l'unité motrice étudiée présente une activité respira-

toire rythmée, la stimulation intensifie les décharges respiratoires et celles-ci peuvent même devenir continues (Fig. 3 *C*), si au contraire l'unité musculaire dans laquelle on insère l'aiguille d'enregistrement est momentanément inactive, la stimulation fait apparaître une activité respiratoire régulière qui peut également devenir continue lorsque la stimulation se fait plus intense (Fig. 3 *B*).

Ces effets pourraient s'expliquer par une convergence d'influx toniques et respiratoires au niveau des motoneurones intercostaux. Lorsque les muscles intercostaux ne présentent pas d'activité tonique, il existe malgré tout, au niveau des motoneurones spinaux, un apport tonique facilitateur qui peut être accru on réduit sous l'effet de la stimulation cérébelleuse ou de réflexes posturaux (8). Celui-ci permet le passage, en périphérie, d'influx subliminaires en provenance du centre respiratoire. Si, par une stimulation ipsilatérale du cervelet ou un réflexe postural, on supprime cet apport tonique facilitateur, les influx respiratoires restent subliminaires et ne parviennent plus aux muscles intercostaux (Figs. 1 *A* ; 3 *A*, *D*, *E*, *F*). Si, au contraire, on l'augmente, soit par la stimulation contralatérale du cervelet, soit par un réflexe postural, on peut faire apparaître, au niveau des muscles intercostaux une activité respiratoire (Fig. 3 *B*, *D*, *E*, *F*), qui peut même devenir tonique (Figs. 1 *B* ; 3 *C*).

Quant à l'activité inspiratoire du muscle diaphragme, nous avons pu nous convaincre, au cours d'une deuxième série d'expériences, que, dans nos conditions d'expérimentation, la stimulation du lobe antérieur du cervelet n'avait aucune action sur elle, pas plus que les réflexes posturaux d'ailleurs.

## Discussion

Nous avons vu, que la stimulation électrique des lobules IV et V du cervelet, dans leur région vermienne et paravermienne, région d'où partent également les efférences vers les muscles des membres antérieurs et des épaules (4, 5), inhibe l'activité musculaire de l'hémithorax ipsilatéral, tandis qu'elle l'intensifie dans l'hémithorax contralatéral, et qu'elle n'a, par contre, aucun effet sur le muscle diaphragme. On trouve là l'explication de l'absence d'effet de la stimulation cérébelleuse sur le débit respiratoire puisque le diaphragme n'est pas influencé par elle, et que l'activité musculaire de chaque hémithorax subit une influence inverse.

Tous ces résultats sont bien dûs à la stimulation du cortex cérébelleux car le seuil peu élevé (0,5 à 1 V, 300 excitations par seconde) exclut une stimulation des structures profondes; de plus, l'application de cocaïne à 1% sur le cortex cérébelleux, inactivant localement celui-ci, supprime toute action de la stimulation du cervelet sur les muscles intercostaux.

Il nous reste donc à examiner par quelles voies nerveuses la stimulation du cervelet agit sur les muscles intercostaux, et comment elle le fait.

En ce qui concerne les voies d'action possibles, il est probable que les effets opposés observés dans les deux hémithorax ne résultent pas d'une influence de la stimulation cérébelleuse sur le centre respiratoire bulbaire. On conçoit mal, en effet, qu'en excitant le cervelet, on inhibe l'activité de la moitié ipsilatérale du centre respiratoire, et stimule simultanément celle de la partie contralatérale, alors que les deux hémicentres respiratoires bulbaires sont fonctionnellement interconnectés au niveau de la ligne médiane (11, 12). De plus, l'absence de modifications de l'activité diaphragmatique, au cours de la stimulation cérébelleuse, semble bien exclure toute participation du centre respiratoire dans nos expériences.

Il faudrait donc admettre que nos résultats sont dûs à une influence du cervelet sur le motoneurone spinal des muscles intercostaux. Il se peut, très vraisemblablement, que la stimulation du cervelet inhibe l'activité des motoneurones intercostaux ipsilatéraux et facilite celle des motoneurones contralatéraux. Cette action serait donc similaire à celle qu'elle exerce sur les motoneurones des muscles extenseurs des membres, et il serait logique que la stimulation influence les motoneurones intercostaux par les mêmes voies, que celles qui agissent sur les motoneurones des muscles extenseurs. En ce cas, les influx nerveux engendrés par la stimulation du cervelet atteindraient d'abord les divers centres toniques du bulbe et du tronc cérébral, lesquels influenceraient à leur tour les motoneurones spinaux, soit directement, soit en agissant sur le circuit proprioceptif musculaire. Les motoneurones du diaphragme, par contre, ne sont pas influencés par la stimulation cérébelleuse dans nos conditions d'expérience.

Quant à l'influence exercée par la stimulation cérébelleuse sur l'activité respiratoire des muscles intercostaux, elle résulterait d'une action indirecte; nous avons vu que la stimulation cérébelleuse agit sur le tonus postural de la cage thoracique en modifiant l'activité

tonique des muscles intercostaux; d'autre part, nous avons été amenés à admettre qu'en modifiant l'apport d'influx toniques aux motoneurones intercostaux, le cervelet facilite ou empêche ces derniers de répondre aux sollicitations du centre respiratoire. De fait, les muscles intercostaux n'ont une activité respiratoire efficace que s'il existe un apport tonique optimal au niveau des motoneurones de ces muscles. Si cet apport est réduit, l'activité respiratoire disparaît et les muscles intercostaux sont relâchés; par contre si l'apport tonique est exagéré, ces muscles deviennent rigides et il n'y a pas davantage de mouvements respiratoires. On comprend donc comment la stimulation du cervelet influence la respiration intercostale: elle agit sur le tonus postural des muscles intercostaux comme sur celui des muscles extenseurs, et c'est en modifiant ce tonus qu'elle influence l'activité respiratoire de ces muscles.

## Résumé

1. Chez le chat décérébré, ayant une respiration spontanée et normale les lobules IV et V du cervelet, région somatotopique du bras et de l'épaule, influencent l'activité tonique et respiratoire des muscles intercostaux. Par contre, ces structures n'influencent guère le muscle diaphragme.

2. La stimulation faradique du cervelet inhibe l'activité tonique des muscles intercostaux ipsilatéraux, mais l'augmente dans les muscles intercostaux contralatéraux. On peut donc affirmer que la stimulation du cervelet agit sur le tonus postural des muscles intercostaux.

3. La stimulation du cervelet agit non seulement sur l'activité tonique des muscles intercostaux, mais aussi sur leur activité respiratoire. Nos expériences montrent que c'est en modifiant l'apport d'influx toniques aux motoneurones intercostaux, que le cervelet facilite ou empêche ces derniers de répondre aux sollicitations du centre respiratoire.

4. Le cervelet agit donc sur le tonus postural des muscles intercostaux comme sur celui des muscles extenseurs, et c'est en modifiant ce tonus que la stimulation du cervelet influence l'activité respiratoire des muscles intercostaux.

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# ANALYSES

R. G. Harrison, *A textbook of human embryology*. Blackwell's, Oxford, xi-244 pp., 42 s.

The rapid progress of research in recent years has led many teachers to reexamine their offerings, with a view to seeing whether traditional material can be curtailed in order to allow more time for study of newer findings and concepts. Dr. R. G. Harrison's textbook represents an attempt to condense and simplify the conventional subject matter of human embryology, primarily for the benefit of medical students.

The book is arranged in the familiar fashion, beginning with the reproductive systems and proceeding through fertilization and formation of the fetus. Short chapters on the pituitary and adrenal glands, and on regeneration, are the only novelties. The text is admirably clear; though always brief, it emphasizes dynamic and functional considerations. Wherever possible the author points out the consequences of developmental pattern on adult anatomy, particularly where teratological effects are apt to occur. In keeping with the intent, many of the illustrations are extremely diagrammatic. But there are also numerous photomicrographs, most of which are of exceptionally good quality. The chapters are provided with short bibliographies that are, with a few exceptions, quite up to date.

On the whole the book is readable and stimulating. Of course the effort to compress a great deal of descriptive and experimental material into a small compass is bound to lead to some superficiality and even downright incorrectness: for example, it is a little disconcerting to read, in 1960, that the chordamesoderm produces « organizers » that are « lipoidal ». Nevertheless, for students who have access to good descriptive material in the laboratory, and are willing to do some supplementary reading, Dr. Harrison's book will provide a most useful introduction to human embryology. Certainly it presents the subject as a growing body of dynamic and important information.

F. Moog

W. E. Adams, *The comparative morphology of the carotid body and carotid sinus*. C. C. Thomas, Springfield, Ill., 1958, pp. 272, L. 4.

Although several important monographs on the physiology of the carotid reflexogenic area have been available for some time, the lack of a comprehensive account of the comparative morphology of this region was deeply felt.

This gap has now been successfully filled by W. E. Adams, whose important research in this field is well known and widely appreciated. The book starts with a historical chapter in which the development of our understanding of morphology and physiology of the carotid body is summarized. Structure, development, innervation and blood supply of the region of the carotid bifurcation in various species of mammals are then described taking into account separately the carotid body and the carotid

sinus. Of particular interest in this section are the data concerning those species in which the internal carotid artery has regressed.

The homologues of carotid body and carotid sinus in birds, reptiles, amphibians and fishes are described and discussed in the second part of the book. Extensive discussion of the significance of the carotid labyrinth of amphibia is included.

The information contained in this monograph is well organized and clearly presented, and the author has made a special effort to report objectively on the different views which have been advanced in the literature on debatable points. The figures are excellent and the biobliography is both accurate and complete.

The book will be very useful to those interested in anatomical, physiological and clinical (especially neurosurgical) problems concerning the region of the bifurcation of the carotid artery.

G. Muratori

*Handbook of Physiology*, Section 1. Neurophysiology. Vol. 1. American Physiological Society, Washington, D. C., 1959, XIII-779 pp.

Within the life time of the present generation of neurophysiologists there has been an extraordinary increase both in the elaborateness of experimental procedures, and in the profusion of published papers. These two factors together contribute to the sense of despair which many active workers experience in their attempt to keep up the literature even in their own fields in any but the narrowest sense. The reading of authoritative and well-balanced review articles is for many the only hope of widening their horizons, but the right authors cannot easily be persuaded to give their energies to this exacting task. The editors of the Handbook of Physiology are to be congratulated in having succeeded for their Volume I in persuading some 30 of the world's most outstanding authorities to contribute a wide and comprehensive selection of chapters upon Neurophysiology.

What the book lacks when compared with the famous Handbooks of the past — and it is inevitable — is a unity of perspective and the grand emergence of general principles. But specialists each dedicated to different disciplines and expert in different techniques cannot be expected to see matters from the same viewpoint. And grand biological principles are entertained more easily where the facts are not so numerous nor measurements so precise as those considered in this volume.

A very pleasing feature in the presentation by some authors is the way in which their own work, though of the highest distinction, has been modestly placed in relation to the great body of scientific matter which they discuss. The unique contributions of some other authors, however, have made this self-effacement less easy to achieve, and actually 60% of all the records reproduced in the Handbook are taken from the particular author's own laboratory as against 40% from the rest of the world.

The bulk of the book is divided into four sections, *a*) nerves and nerve junctions; *b*) brain potentials; *c*) sensory mechanism other than *d*) vision. Section *a*) is perhaps the most impressive for the analytic power and precision which is now being directed to the understanding of the minute and rapid changes which accompany nerve activity. Eccles's introduction takes us in bold, clear strides from membrane structure to synaptic potentials. On the way he briefly describes the Hodkin-Huxley theory of nerve conduction which he finds ...." such an immensely impressive performance that the ionic hypothesis of the nerve fibre must rank as one of the great achievements of biology." This treatment is welcome since the theory, though repeatedly referred to in subsequent chapter, is not discussed elsewhere in the Handbook.

Tasaki develops the physiology of the nerve impulse not only from the squid giant nerve but also from his unrivalled knowledge of the properties

of the node of Ranvier. Gray gives a well-balanced account of generator potentials in sens organs and the way in which impulses are initiated in the sensory nerve. Grundfest's 50-page chapter upon synaptic and ephaptic transmission is exceedingly clearly written and goes far to make intelligible the immense complexity of this subject. Fatt and U. S. v. Euler take up the simpler question of neuroeffector transmission in skeletal and autonomic nerves and treat their subjects with the expertise of the electrophysiologist and pharmacologist respectively.

A very welcome feature is the inclusion in the Handbook of some chapters upon neurophysiology of invertebrates, and when we begin to think we understand the physiology of "neuromuscular transmission" Furshpan's account of the process in arthropods, molluscs and coelenterates comes as a timely reminder of what utterly different mechanism, are employed in animals where only a few neurones are available.

*b*) The subject of brain potentials introduced by Fessard naturally will not admit of such exact treatment. The main features of modern recording techniques and the principles of interpretation of electric records are very clearly set out by K. Frank. Grey Walter develops the fundamental idea of rhythm from physical system and discussed the nature of various E.E.G. rhythms. Chang gives a very careful and critical account of the various features of evoked potentials. O'Leary and Goldring discuss the d. c. potentials of the cerebral cortex, and the physio-pathology of epileptic seizures is treated by Gastaut and Fischer-Williams basically from the clinical approach.

*c*) The section on sensory mechanisms is introduced by Lord Adrian. Autrum them with masterly compression and clarity covers the enormously diverse subject of non-photic receptors in all forms of life below the vertebrate.

In the study of sensory organization in the vertebrate two main problems arise. The first involves the anatomy of the nerve pathways, how impulses from various types of end organ, or subserving various kinds of stimulation diverge into different tracts, connect with varied nuclei, come together again in particular locations and generate or modify electrical activity in special regions of the brain. The second problem is physiological: How does this achieve the organization we observe? Upon the latter no one has anything substantial to say. We cannot measure organization — principally because we are not clear what we need to measure. Engineers were driven to invent the science of thermodymanics in order to study the organization of steam molecules, and we shall have to develop *neurological information theory* if we are to study the organization of the brain. Such theory will probably soon begin to be effective, but at present the study of sensory organization is restricted almost entirely to a description of the anatomy of sense organs and their nerve pathways, and to the patterns of electric activity resulting from specific stimuli.

That this in itself is task enough may be appreciated from Rose and Mountcastle's fine chapter upon touch and kinesthesis. They combine a critical and cautious judgment as to the conclusions which can be drawn from purely anatomical considerations with a stimulating attitude towards the answers obtainable by combining anatomy with suitable electrophysiological techniques. Zotterman treats the subject of thermal sensations going from the psychological approach of Weber, through Bazett's heat measurements in the human skin, and finally explaining much in terms of the single nerve records from his own work with Hensel

The sensation of pain may be elicited by so many kinds of stimuli and has so large a psychological component that experiments on animals bring little conviction unless they correspond with human experience. The chapter by Sweet, therefore, is based upon clinical observations of pain in relation to specific alteration (e.g. section) of nerve pathways, and the comparison of this with behaviour and records from animals in similar situations.

The treatment of taste by Pfaffmann, smell by Adey, and vestibular

sense by Gernandt give in each case good descriptions of the sense organs and nerve records resulting from various kinds of adequate stimulation.

Hallowell Davis's account of excitation of the auditory receptors describes with masterly clarity the transformation of pressure pulses in air into a deformation wave running up the basilar membrane. The mechanical and electrical events in hair cells and their nerves are seen to follow reasonably from this presentation. The central mechanism of audition is treated by Ades mainly from the anatomical aspect with especial attention to the localization of various kinds of auditory function in cat, and primate.

*d*) The section on vision is introduced by Hartline. L. J. and Margery Milne in a compact and comprehensive chapter classify light sensitivity and visual organs in the whole of the invertebrate kingdom. The image-forming properties of the human eye are treated expertly by Fry. Wald describes the properties of the principal visual pigments in the eye and (more exactly) in extracts, and he relates these to the phenomena of vision. His chapter makes the more fascinating reading from the way his facts are displayed as examples of wide-sweeping general principles. Granit in a brief 20 pages proceeds from the structure of the retina through E.R.G. and neurone spikes to the effect of centrifugal control. In a field where he has already written so extensively, he here picks out a few selected topics for special discussion. Bartley faces the complexities in the central mechanisms of vision, and the correspondence and non-correspondence between the neural records and the sensation experienced. Livingston completes the volume with a discussion of the central control of receptors by centrifugal fibres as well as the interactions (mainly inhibitory) between the various input system.

The Handbook opens with perhaps its most attractive chapter, the h[illegible]ical development of neurophysiology by Mary Brazier. This mature and well-documented account traces the emergence of fundamental concepts from classical times to the present day, and is embellished with 40 reproductions from old books and portraits.

The Waverly Press Inc., Baltimore, is to be congratulated upon the high quality of the printing and reproductions.

W. A. H. Rushton

# TABLES DE MATIÈRES

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